Fish fauna structure, feeding ecology and growth of keystone fish species in the Elbe and Odra estuaries

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Chapter 3: Growth patterns of key stones fish species in temperate estuarine ecosystems – a comparison between the Elbe and Odra estuaries

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Chapter 4: Environmental factors shaping fish fauna structure in a temperate mesotidal estuary: Periodic insights from the Elbe estuary across four decades

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Chapter 5: Network-based integration of omics, physiological and environmental data in real-world Elbe estuarine Zander

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Table of Content

Summary

In the Elbe and Odra estuaries essential feeding grounds, nursery areas, spawning grounds and transitional habitats for several fish species are found. With climate change and human activities (e. g. channel management, waste water sewage and nutrient inputs) both estuaries have faced several anthropogenic stressors in the past and in the present. These stressors are likely to have an influence on estuarine ecosystems and their food webs. Keystone fish species play an important role in estuarine food webs. With this regard, in this thesis the feeding ecology and growth of keystone fish species in the Elbe and Odra estuaries were analysed. Additionally, in the Elbe estuary, the structure and stock development of the fish fauna, as well as fish health, were evaluated based on stow net catch data and tissue-specific stress responses of the holobiont. Analyses on feeding and growth of the four keystone fish species zander *Sander lucioperca* (Linnaeus, 1758), smelt *Osmerus eperlanus* (Linnaeus, 1758), ruffe *Gymnocephalus cernua* (Linnaeus, 1758) and flounder *Platichthys flesus* (Linnaeus, 1758) were carried out, as these species occur in high numbers throughout both estuaries and play key roles in the respective aquatic food webs.

The food webs in estuarine ecosystems are important biological indicators, therefore the feeding ecology of the four keystone fish species was studied by stomach content analyses (**chapter 2**). The most important prey of zander in both estuaries were fish and mysids. For smelt amphipods were the most important prey in the Elbe estuary, whereas in the Odra estuary smelt mainly consumed mysids. The most important prey of ruffe in the Elbe estuary were also amphipods, while annelids (lower estuary) and insect larvae (upper estuary) were the dominant prey in the Odra estuary. In the Elbe estuary, flounder fed mainly on copepods, whereas in the Odra estuary they preferred bivalves. In the Elbe estuary higher dietary overlaps were found, particularly between smelt and ruffe, zander and ruffe, as well as zander and smelt. By comparing the present diets of zander, smelt and ruffe from the Elbe estuary with previous studies (1990s), it was noticeable that the consumption of amphipods increased, while mysids and copepods decreased in their diets. These shifts indicated a restructuring of the food web, likely driven by environmental changes, which highlights the vulnerability of estuarine ecosystems.

In **chapter 3** we aimed to analyse the growth patterns of the four keystone fish species to identify regional differences from both estuaries and to compare present findings with those from past studies. Age determination on hard structures (otoliths, scales, opercula) and length measurements were carried out. This allowed the determination of size ranges of the individual age groups, which were used to calculate the parameters of the von Bertalanffy growth function. The asymptotic lengths of smelt, ruffe, and flounder were larger in the Elbe estuary compared to the Odra estuary, whereas their growth coefficients were smaller in the Elbe. The growth of zander, smelt and ruffe from the Elbe

estuary were inferior compared to previous studies. The growth of juvenile zander in the Elbe estuary showed a noticeable reduction in the maximum turbidity zone located in the middle estuary. Slower growth at these sites appears to be linked to reduced fish prey intake, as juvenile zander relied more on invertebrates like amphipods, mysids, and decapods. Flounder from the Odra estuary showed smaller asymptotic length compared to other studies from the Baltic, but had a higher growth coefficient in contrast to studies from the Pomeranian Bay and Gulf of Gdańsk. Our findings indicate a connection between environmental changes and fish growth via changes in feeding behaviour, especially in the Elbe estuary.

We created a periodic time series over the last four decades (1984-2022), combining fish species densities data from stow net catches alongside with environmental data in the Elbe estuary. In **chapter 4**, we showed that the fish fauna of the Elbe estuary has become more similar in guild structure to that of macrotidal estuaries in Europe, with a relative increase in marine-estuarine opportunists and a decrease in diadromous species. Due to an improvement of the water quality in the 1990s, fish densities, especially of smelt, increased until 2010. However, anthropogenic hydromorphological interventions, have led to an increase of suspended particular matter until 2022. Together with a reduced river runoff and poor oxygen condition, these factors acted as stressors for fishes in the estuary. In 2021-2022 mean fish densities dropped by over 91 % compared to 2009-2010. This decline was mainly driven by a reduction of the key species smelt, alongside decreases in twaite shad *Alosa fallax* (Lacepede, 1803), flounder, ruffe, common bream *Abramis brama* (Linnaeus, 1758), and other species. In contrast, densities of marine-estuarine opportunist species such as herring *Clupea harengus* Linnaeus, 1758 and whiting *Merlangius merlangus* (Linnaeus, 1758) increased. Overall, this time-series provided insights into the strong impact of human intervention in estuarine ecosystems.

In **chapter 5**, we integrated tissue specific gene expression data from fish host (gill and liver RNAseq) and 16S rRNA metabarcoding of gill mucus microbiota with physiological and abiotic parameters to assess the health of juvenile zander along the Elbe estuary. Liver specific gene expression patterns in fish from highly turbid areas in the middle estuary indicated starvation, which aligned with the compromised body condition. The gill microbiome derived from the freshwater area with local occuring oxygen minimum zones was dominated by potentially pathogenic taxa, including *Shewanella*, *Acinetobacter*, *Aeromonas* and *Chryseobacterium*. Their presence was found alongside with strong immune responses in the host gill tissue and increased energy demand in the liver tissue, supporting a potentially pathogenic role. Together, these results demonstrate how physiological and microbiome responses in estuarine fish can reflect the cumulative impact of abiotic stressors, which are likely to intensify under ongoing climate change.

Zusammenfassung

Im Elbe- und Oderästuar befinden sich wichtige Nahrungs-, Aufwuchs- und Laichgebiete sowie Wanderungsgebiete für verschiedene Fischarten. Beide Ästuare wurden sowohl in der Vergangenheit als auch in der Gegenwart durch anthropogene Stressoren wie Klimawandel, Gewässerausbau, Abwasserentsorgung und Nährstoffeinträge beeinflusst, was vermutlich Auswirkungen auf die Ökosysteme und deren Nahrungsnetze hatte. Schlüsselfischarten spielen eine wichtige Rolle in den Nahrungsnetzen von ästuarinen Ökosystemen. In diesem Zusammenhang ist das Ziel dieser Arbeit die Analyse der Nahrungsökologie und des Wachstums wichtiger Fischarten in den Ästuaren von Elbe und Oder. Es wurden Analysen zur Ernährung und zum Wachstum der vier Schlüsselfischarten Zander *Sander lucioperca* (Linnaeus, 1758), Stint *Osmerus eperlanus* (Linnaeus, 1758), Kaulbarsch *Gymnocephalus cernua* (Linnaeus, 1758) und Flunder *Platichthys flesus* (Linnaeus, 1758) durchgeführt, da diese Arten in beiden Ästuaren häufig vorkommen und eine Schlüsselrolle spielen. Darüber hinaus wurden im Elbeästuar die Struktur und Bestandsentwicklung der Fischfauna sowie die Fischgesundheit anhand von gewebespezifischen Stressreaktionen beurteilt.

Nahrungsnetze in ästuarinen Ökosystemen haben eine biologische Indikatorfunktion. Aus diesem Grund wurde die Nahrungsökologie von vier Schlüsselfischarten durch Mageninhaltsanalysen untersucht (**Kapitel 2**). Die wichtigste Nahrung des Zanders in beiden Ästuaren waren Fische und Mysiden. Die wichtigste Nahrung des Stints im Elbeästuar waren Amphipoden, während Stinte im Oderästuar bevorzugt Mysiden fraßen. Die wichtigste Nahrung des Kaulbarschs im Elbeästuar waren ebenfalls Amphipoden, während im Oderästuar Anneliden (unteres Ästuar) und Insektenlarven (oberes Ästuar) die wichtigste Nahrung darstellten. Die wichtigste Nahrung von Flunder aus dem Elbeästuar waren Copepoden, wohingegen sie im Oderästuar Muscheln bevorzugten. Im Elbeästuar wurden größere Nahrungsüberlappungen festgestellt, insbesondere zwischen Stint und Kaulbarsch, Zander and Kaulbarsch sowie Zander and Stint. Beim Vergleich der rezenten Ernährung von Zander, Stint und Kaulbarsch aus dem Elbeästuar mit früheren Studien (1990er Jahre) fiel auf, dass der Verzehr von Amphipoden zunahm, während Mysiden und Copepoden in ihrer Ernährung zurückgingen. Diese Verschiebungen deuten auf eine Umstrukturierung des Nahrungsnetzes hin, welche wahrscheinlich auf Umweltveränderungen zurückzuführen sind.

In **Kapitel 3** war das Ziel, Wachstumsmuster von den vier Schlüsselfischarten zu analysieren, um regionale Unterschiede in beiden Ästuaren zu identifizieren und aktuelle Ergebnisse mit denen früherer Studien zu vergleichen. Es wurden Altersbestimmungen an Hartstrukturen (Otolithen, Schuppen, Opercula) sowie Längenmessungen durchgeführt. Dadurch konnten Größenbereiche der einzelnen Altersgruppen ermittelt werden, welche zur Berechnung der von Bertalanffy-Wachstumsfunktion herangezogen wurden. Die asymptotischen Längen von Stint, Kaulbarsch und

Flunder waren im Elbeästuar größer als im Oderästuar, wohingegen ihre Wachstumskoeffizienten in der Elbe kleiner waren. Das Wachstum von Zander, Stint und Kaulbarsch aus dem Elbeästuar war im Vergleich zu früheren Untersuchungen geringer. In der Zone mit der maximalen Trübung im mittleren Elbeästuar zeigten juvenile Zander ein deutlich geringeres Wachstum. Langsameres Wachstum in diesem Bereich scheint mit einem geringeren Anteil von Fischen in der Nahrung zusammenzuhängen, da juvenile Zander dort mehr Wirbellose wie Amphipoden, Mysiden und Dekapoden fraßen. Flundern aus dem Oderästuar zeigten im Vergleich zu anderen Studien aus der Ostsee eine geringere asymptotische Länge, hatten aber im Gegensatz zu Studien aus der Pommerschen Bucht und der Danziger Bucht einen höheren Wachstumskoeffizienten. Unsere Ergebnisse deuten darauf hin, dass Umweltveränderungen, insbesondere im Elbeästuar, das Fischwachstum beeinflusst haben, was den Zusammenhang mit einem veränderten Fressverhalten verdeutlicht.

Für **Kapitel 4** wurde eine Zeitreihe von 1984 bis 2022 zusammengestellt, in der Daten zur Dichte von Fischarten aus Hamennetzfängen mit Umweltdaten im Elbeästuar kombiniert wurden. Die Gildenstruktur der Fischfauna im Elbeästuar ähnelt zunehmend der makrotidaler Ästuare Europas, mit einem relativen Anstieg der marine-ästuarinen Opportunisten und einer Abnahme der diadromen Arten. Einhergehend mit der Verbesserung der Wasserqualität in den 1990er Jahren stieg auch die Fischdichte, insbesondere die des Stints im Elbeästuar, bis 2010 an. Die bis 2022 durchgeführten hydromorphologischen Eingriffe haben zu einem Anstieg der Schwebstoffkonzentrationen geführt. Verminderter Oberflächenabfluss und niedrige Sauerstoffwerte wirkten als Stressoren für Fische im Elbeästuar. Von 2009–2010 bis 2021–2022 sank die durchschnittliche Fischdichte um über 91 %. Dieser Rückgang war hauptsächlich auf einen Rückgang der Arten Stint, Finte (*Alosa fallax*) (Lacepede, 1803), Flunder, Kaulbarsch, Brassen *Abramis brama* (Linnaeus, 1758) sowie weiterer Arten zurückzuführen. Im Gegensatz dazu nahm die Dichte von marine-ästuarinen Opportunisten wie Hering *Clupea harengus* Linnaeus, 1758 und Wittling *Merlangius merlangus* (Linnaeus, 1758) zu. Insgesamt lieferte diese Zeitreihe Einblicke in die Auswirkungen menschlicher Eingriffe auf das Ökosystem Elbe.

In **Kapitel 5** wurden gewebespezifische Genexpressionsdaten von Fischen als Wirt (Kiemen- und Leber-RNAseq) und 16S-rRNA-Metabarcoding von Kiemenschleim mit physiologischen und abiotischen Parametern assoziiert, um die Gesundheit juveniler Zander entlang des Elbeästuars zu bewerten. Genexpressionsmuster in der Leber von Fischen aus der Zone maximaler Trübung im mittleren Ästuar zeigten Hinweise auf Nahrungsmangel, was mit einem verringerten Korpulenzfaktor einherging. Das Mikrobiom der Kiemen von Fischen aus der Sauerstoffminimumzone (Süßwasser) wurde von potenziell pathogenen Taxa dominiert, darunter *Shewanella*, *Acinetobacter*, *Aeromonas* und *Chryseobacterium*. Ihr Vorkommen wurde zusammen mit starken Immunantworten im Kiemengewebe des Wirts und einem erhöhten Energiebedarf im Lebergewebe festgestellt, was ihre potenzielle pathogene Rolle unterstützt. Zusammengenommen zeigen diese Ergebnisse, wie physiologische und mikrobiologische Reaktionen von Fischen in Ästuaren die kumulativen Auswirkungen abiotischer Stressfaktoren widerspiegeln können, welche sich im Zuge des anhaltenden Klimawandels wahrscheinlich verstärken werden.

Chapter 1 General Introduction

1.1 Structure and types of estuaries

Definition and zones of an estuary

The definition of “estuaries” differs between geologists, hydrologists and biologists (Blaber, 2000). Day (1981) and Blaber (1997) defined estuaries as semi-enclosed coastal water bodies, that have a continuous or periodical connection to the open sea in which sea water is diluted with freshwater (Figure 1.1). They represent a transition zone between freshwater and marine ecosystems. A salinity gradient is formed by the mixing of riverine freshwater and marine waters. Not only the river mouth areas in the Northeast Atlantic and North Sea, which are influenced by the tide, can be defined as estuaries, but also bays, lagoons and river mouths in the intercontinental (e.g. European Mediterranean) and intracontinental seas (e. g. the Baltic Sea) which are influenced only little by the tides (Nellen and Thiel, 1995; Thiel, 2011). Due to its pronounced salinity gradient, with a decreasing salinity from east to west, the entire Baltic Sea can be considered as an estuary (Fennel, 1995; Matthäus, 1995; Thiel, 2011).

Generally, estuaries can be classified into different zones (Snedden et al., 2013): the upper, middle and lower estuary (Figure 1.1). The upper estuary is characterized by freshwater and is typically influenced by river discharge. The middle estuary is the transition zone where the maximum mixing of freshwater and saltwater occurs, creating a brackish environment with fluctuating salinity levels. In the lower estuary, the marine influence is increased by more intrusion of coastal saltwater. In the mixing zone of a tidally influenced estuary, a maximum turbidity zone (MTZ) is found at salinities from 1 to 5 ppt (Day et al., 2012).

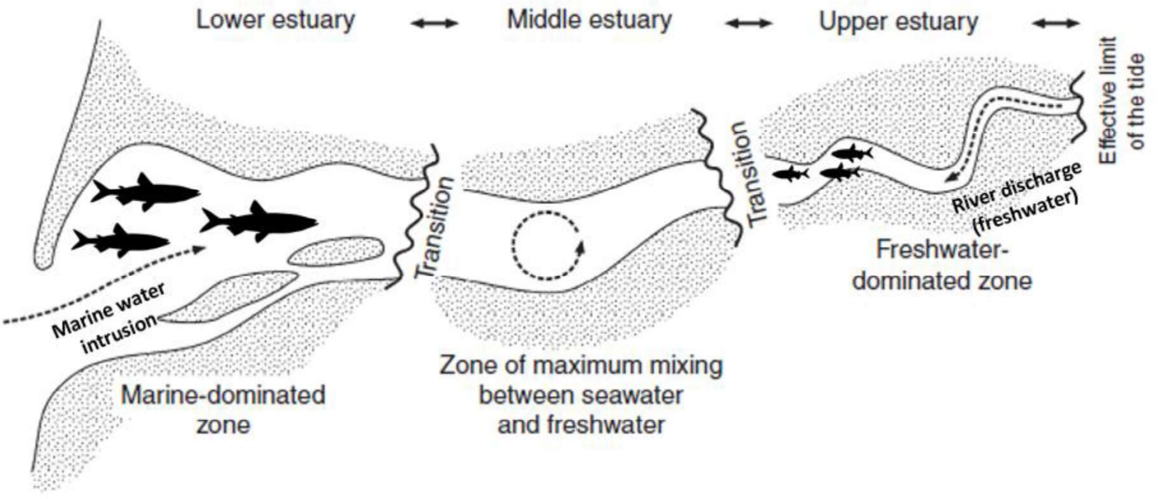

Figure 1.1: Schematic drawing of the ‘standard’ estuary. Modified from Perillo et al. (1999) and Whitfield and Elliott (2011).

Chapter 1 – Introduction

Types of estuaries

Estuaries can be classified according to their geomorphology into distinctive groups: (1) coastal plain estuaries (Figure 1.2), (2) deltaic estuaries, (3) lagoons (or bar-built estuaries, Figure 1.2), (4) fjords and (5) tectonically caused estuaries (Pritchard, 1952; Dyer, 1973; Snedden et al., 2012). Coastal plain estuaries (e. g. Chesapeake Bay, Elbe) consist of a funnel-shaped channel, usually not deeper than 20 m either bordered by broad shallow flats of salt marshes. They were formed during the last eustatic sea-level rise, when river valleys became increasingly more flooded by melting glaciers (Snedden et al., 2012). In deltaic estuaries (e.g. Mekong, Mississippi) the main channel branches into multiple side channels, forming a dendric network of distributaries. These estuaries are generally shallow (< 5 m) and can harbour expansive fresh-, brackish- and salt marshes and mangroves. The seasonal variation in discharge is induced either due to winter snowmelt or tropical wet season precipitation, which leads to overbank flooding transporting nutrients within the system (Snedden et al., 2012). According to Islam and Gnauck (2009) delta estuaries are among the most productive but fragile estuaries worldwide. Lagoons (e. g. Greifswald Bodden, Odra Lagoon) have a larger area of open water, compared to the other estuary types with physical constraints such as narrow channels, strong tidal currents, or confined geomorphology. They are usually shallow with no extensive tidal influences and physical processes such as mixing of fresh- and saltwater which is induced by winds (Snedden et al., 2012). Fjord estuaries are steep walled and U-shaped valleys, that were glacially carved in high latitude environments. They are geologically young and evolved over a relative short time span. Fjord estuaries express ranges in deglaciation from either completely glacier-filled to completely deglaciated (Etherington et al., 2007). Often fjords have a sill at the opening to the sea side, which limits water exchange between the deeper parts of the fjord and the open ocean. Fjords are among the types of estuaries that can have higher water depth, reaching down to more than 1.000 m below sea level (e.g. Manzetti et al., 2010). At the surface, the currents are often directed out of the fjord by freshwater runoff, since above the sill depth water can freely move in and out of the fjord (Ribergaard et al., 2008). Tectonic estuaries were formed by geological events such as earthquakes, fractures or generated deformations on the Earth's crust in coastal areas. This allows the formation of hollow basin below the sea level, which is then flooded by marine water (Nebra, 2015).

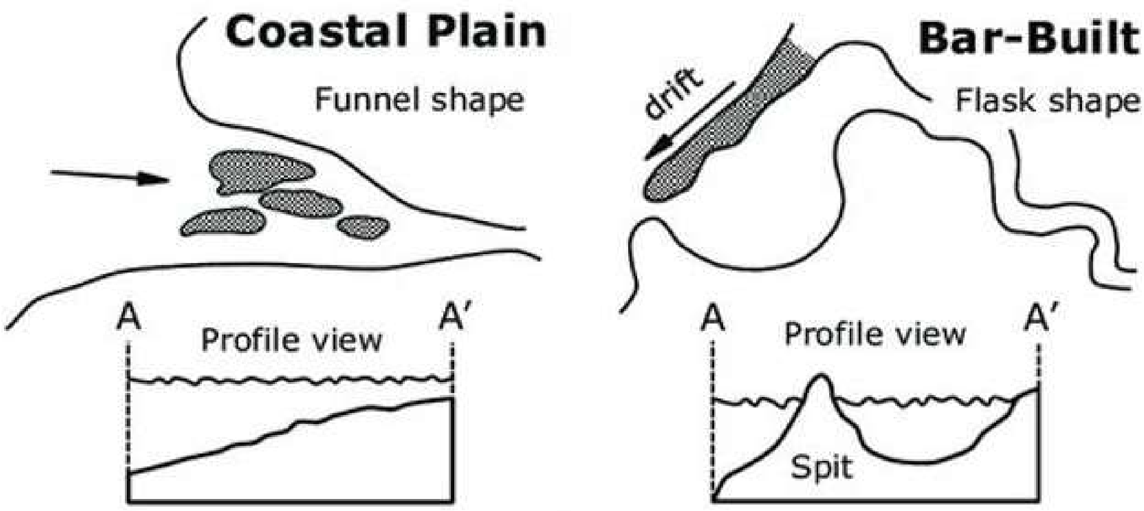

Figure 1.2: Longitudinal and profile view of coastal plain and bar-built estuary types. Modified from Nebra (2015).

The tidal range, which is the difference between high and low tide, varies around the world. Hayes (1975) adopted an estuarine classification system that is based on tidal range into microtidal (< 2 m), mesotidal (2 – 4 m) and macrotidal (> 4 m) categories (Whitfield and Elliott, 2011). For instance, the tidal range in the semi enclosed Mediterranean is small with approximately half a meter, resulting in narrow intertidal areas. On the contrary due to the shape of the coastline the Bay of Fundy in Canada has a mean tidal range of 12 m (Dipper, 2022).

Environmental conditions and anthropogenic influences in estuaries

Estuarine ecosystems display highly dynamic habitats, which are characterized by varying abiotic factors such as salinity, turbidity, discharge regime as well as the ecologic master factors temperature and oxygen content (Thiel and Potter, 2001; Elliott and McLusky, 2002). These changes in abiotic factors are driven by seasonal fluctuations, weather patterns and tidal dynamics and therefore present challenging environments for biota due to its dynamic nature. According to Day (2012) several abiotic factors shape the unique characteristics of an estuary:

1. Protection against marine forces – by the degree of shielding from direct wave action and tidal influences.
2. Freshwater input and sediment load – by the amount of freshwater, alongside with dissolved and suspended materials, that affects water composition and productivity.
3. Water circulation – by riverine and tidal currents as well as winds that influence the circulation. Also, the tides strongly influence estuarine hydrodynamics and biological processes.
4. Estuary depth – in shallow estuaries there is a stronger interaction between the water column and the bottom. Therefore, nutrients from sediments can be released to fuel phytoplankton growth in surface waters.

5. **Sharpness and pattern of the salinity gradient** – the transition from marine to freshwater influences circulation affects the biological processes and organisms
6. **Rapid geomorphological changes** – Sandbanks, mudflats, and wetlands form, degrade, and shift, with sediment deposition are shifted by the currents.

In tidal estuaries, the main physical drivers are the tides, which determines the intrusion of marine water. With increasing tidal range, the mixing zone migrates further into the upper areas of the estuary (Lang, 1990). Another physical driver is the quantity of riverine freshwater discharge, which also determines the salinity distribution and stratification. River discharge dynamics often underly seasonal fluctuations by either high discharge events during early spring induced by higher rates of precipitation or snow melting, or by droughts during summer when river discharge is reduced.

The salinity gradient is one of the key features of estuarine ecosystems and substantially affects the species composition and biogeochemical processes (Cloern et al., 2017). The lower estuary, characterized by highest salinity concentrations, is dominated most by marine species. In upstream direction, riverine freshwater discharge becomes more prominent in the estuary, resulting in an increase of freshwater species. There are estuaries with different types of mixing fresh- and saltwater, which is dependent on the respective hydrology. The process of mixing fresh- and saltwater in an estuary is neither uniform nor steady, as the ratio of fresh- and saltwater and the river-cross section increase seawards. In a salt wedge estuary, the water column is stratified, with a halocline that separates the riverine water and a wedge of saltwater on the bottom layer, while in a well-mixed estuary no halocline is found (Kumar et al., 2015).

Dissolved oxygen (DO) can enter the water column through photosynthesis by primary producers as well as atmospheric diffusion, which can be enhanced by winds or increased surface agitation (Downing and Truesdale, 1955). Bacteria and other decomposing organisms can reduce DO levels in estuaries, since they consume oxygen while breaking down organic matter. Oxygen depletion can occur in estuaries when a high abundance of primary producers dies and decomposes as a result of eutrophication (Conley et al., 2011; Schöl et al., 2014; Tiedke et al., 2014; Weinke and Biddanda, 2017). The DO concentration found in an estuary are also coupled to freshwater discharge and water circulation (Kauppila et al., 2003).

Turbidity is an indicator for the transparency of the water and is formed by the presence or absence of suspended solids that are in the water column. With higher turbidity the amount of light that penetrates the water is reduced, which leads to light limitation for primary producers and inferior visibility for predators like fish (Brown, 1984; Rodrigues et al., 2023). In tidal estuaries, a maximum turbidity zone (MTZ) can be found typically at the boundary (mixing zone) between fresh- and marine waters, where the concentration of suspended particulate matter is highest. The flood tide, which is generally shorter but more energetic than the ebb tide, resuspends sediments and transports them landward. As a result, the sediment transport is typically larger during flood tide, compared to the ebb tide (Allen et al., 1980; Mitchell, 2013). The location of the MTZ strongly depends on the river discharge. Uncles et al. (1999) showed in their 3-year measurements in the Humber-Ouse estuary, that the drought of summer 1995 shifted the MTZ more than 60 km upstream compared to its position in the previous winter. Therefore, the MTZ in macrotidal estuaries is found more upstream compared to meso- and microtidal estuaries.

Despite being important ecosystems, estuaries worldwide are heavily influenced by human activities (Ahlhorn, 2009) through impacts from industrial sewage, dyke construction, deepening of navigation channels and disposal of sediments (e.g. Barbier et al., 2011; Drabble, 2012; Wetzel et al., 2014). The stressors on estuarine ecosystems are intensified by climate changes (van Beusekom et al., 2018) on a long-term perspective (Ducrotoy et al., 2019). All in all, these anthropogenic stressors have influenced species richness, biomass, genetic structure and food webs in estuaries (Gilarranz et al., 2016). By dam constructions, habitat connectivity can be destroyed and life-cycle closure in marine-estuarine organisms may be endangered (Whitfield et al., 2023). Officer (1980) stated that when hydrodynamic alterations occur quickly relative to biological, geological and chemical transformation, they become the dominant controlling factors in many ecological processes in estuaries.

Climate change, which includes rising temperatures, sea level rise and increased risks of floods and droughts, increases the risk of abrupt nonlinear shifts in estuarine ecosystems altering the ecosystem composition, function, biodiversity and productivity (Ducrotoy et al., 2019). Reponses to climate change may vary from estuary to estuary worldwide, because estuarine surface temperatures are influenced by a complex combination of solar radiation, air temperature, ocean temperature, water exchange and winds (Brown et al., 2016). By sea level rise, the volume of tidal water entering the estuarine channel lead to tidal intrusion, amplitude and current velocities in the upper estuarine channel (Holleman and Stacey, 2014). In temperate estuaries, rising water temperatures are expected to favour spreading of southern species (lusitanian), while species adapted to colder water will be negatively affected by shifts in seasonal cycles (e.g. spawning periods, larval occurrence) and changes of growth condition, particularly due to altered food availability (Thiel, 2011). Polte et al. (2021) described phenological mismatches, when the timing of the phyto- and zooplankton bloom is misaligned with the hatching of fish larvae. Rising water temperature can affect oxygen levels in estuaries. Firstly, warmer water cannot hold as much oxygen as colder water (Allen, 1955) and secondly the microbial metabolisms increases, which can cause hypoxia. There has been recognition by several authors, that climate change impacts hypoxia in estuarine and coastal waters (Altieri and

Gedan, 2015; Bendtsen and Hansen, 2013; Claret et al., 2018; Justić et al., 2003; Meier et al., 2011; Ni et al.; 2019). Extreme drought events in the past have led to decline of freshwater fish species, reduction of resident fish populations and increases of marine stragglers in Iberian estuaries (Martinho et al., 2007; Nyitrai et al., 2013). This significant decline in estuarine productivity during drought years may have also impacted marine fish stocks through a reduced recruitment and export (Dolbeth et al., 2008).

Biguino et al. (2024) concluded that despite the increase in climate change research since 2000, only 9.3 % of marine climate change studies focused on estuaries, of which only 1.13 % were in situ studies. This highlights the significant gap in observation data and the need for extensive in situ monitoring to better understand and address climate change effects in estuarine environments.

Elbe and Odra estuaries

The Elbe estuary is strongly influenced by tides and is classified as mesotidal coastal plain estuary without a marked halocline (Thiel and Potter, 2001; Eick and Thiel, 2014; Pein et al., 2023). It reaches from the upper boarder at the weir Geesthacht to the river mouth close to the city Cuxhaven, where it enters into the southern North Sea. This estuary has been shaped by a long history of human alteration (Bergemann et al., 1996; Kerner, 2007; Amann et al., 2012; Pein et al., 2021) and therefore shares fate with estuaries worldwide (Pein et al., 2023). Since the mid nineteenth century the Elbe estuary has experienced channelization for shipping and an increase in communal, industrial and agricultural sewages as well as water abstraction for power plant cooling (Thiel et al., 2003; Kerner, 2007; Eick and Thiel, 2014). The alteration of the hydromorphology of the main channel experienced strong influences on the river runoff dynamics, tidal range and current velocities. In the Hamburg port area (100 km upstream of the river mouth), the tidal range doubled from 1.9 m to 3.8 m over the last century (Hein et al., 2021). Recently, a reduction of the sub- and intertidal shallow water areas occurred due to construction and sedimentation processes, especially in the freshwater area (Scholle and Schuchard, 2020). At the same time, in the Hamburg port area (freshwater zone) oxygen deficiencies were reported on regular basis during summer months (Riedel-Lorjé and Gaumert, 1982; Thiel, 2011; Eick and Thiel, 2014; Scholle and Schuchard, 2020). However, the Elbe estuary still harbours ecological populations and habitats, that are internationally important, like a number of other temperate estuaries (Thiel, 2011; Ducrotoy et al., 2019)

The Odra estuary, which is located in north-east Germany drains to the Baltic Sea and has very low tidal influences with a tidal oscillation below 20 cm (Medvedev et al., 2013). It can therefore be classified as microtidal lagoon estuary. The Odra estuary can be assigned into three distinctive sections:

upper section (Odra Lagoon: OL) with salinities ranging between 1 and 3 PSU, the middle section (Greifswald Bodden: GB) with a salinity between 6 and 7 PSU and the lower section (Pomeranian Bay: PB) with a salinity between 5 and 8 PSU (Gruszka, 1999; Schernewski, 2011). The river bottom of the Odra estuary is covered mostly by muddy and sandy substrates. In the Odra Lagoon and Greifswald Bodden, the bottom structures are dominated by fine-grained sediments, while the Pomeranian Bay is dominated by sands (Wypych, 1980; Kube et al., 1996; Gruszka 1999). The Odra Lagoon is partly enclosed and its water exchange with the Pomeranian Bay is highly depended on factors such as wind conditions, sea levels and the discharge from the Odra river. The largest water exchange occurs through the Świna (60 -70 %), while through Peenestrom and Dziwna approximately 15 – 20 % water exchange occurs (Jasińska and Massel, 2007; Kowalewska-Kalkowska, 2021). Environmental conditions such as salinity and oxygen concentration are prone to seasonal variation. During spring the salinity levels typically decrease due to increased river discharge and during autumn the salinity increased when influx of more saline waters occurs due to storms. In the Odra Lagoon, supersaturation of the surface water levels during summer month occur regularly (Chojnacki and Tyluś, 2013), which is accompanied by increased turbidity and temporal occurrences of hypoxia (Radziejewska and Schernewski, 2008; Schernewski, 2011; Friedland et al., 2012; Pein and Staneva, 2024). The Baltic Sea has a long history of eutrophication (Voss et al., 2011; Pein and Staneva, 2024), that results in regular blooms of microalgae, including cyanobacteria during summer months.

1.2 Fish in European estuaries

Species numbers and biogeographic affinities

According to Elliott and Hemmingway (2002) a total of 255 fish and lamprey species (consecutively referred as fish species) were found within 26 European estuaries. Franco et al. (2008), who included a larger number of estuaries in the Mediterranean, named 317 fish in 38 estuaries, inhabiting between 15 % and 18.6 % of the European fish species (Thiel, 2011). The average species number found in European estuaries is 56 fish species, ranging from 22 fish species in the Solway estuary and 119 fish species in the Thames estuary (Thiel, 2011).

Marine, estuarine and diadromous fish species in European estuaries can be subdivided by their biogeographic affinity (boreal, lusitanian, atlantic) (Jiming, 1982; Dulvy et al., 2008; Engelhard et al., 2011; Sarrazin et al., 2021). Boreal fish species, occur predominantly between the Norwegian Sea and The British Islands, which have preferences for colder water temperatures (e. g. *Osmerus eperlanus*). Lusitanian fish species, have a distribution range between the northwest North Sea (British Islands) and die Iberian Peninsula/North Afrika and prefer warmer temperatures (e.g. *Merlangius merlangus*).

Atlantic fish species, occur predominantly in the Atlantic Ocean and around the European coasts (e.g. *Salmo salar*). Freshwater fish species can be further grouped into two zoogeographical categories (Leroy et al., 2019): Palearctic (fish species that have European, Siberian and East-Asian distribution) and Nearctic (introduced from North America). Spatial and temporal variation in the fish fauna composition, can be distinguished by the guild composition. A longitudinal change in the fish community in upstream direction, corresponds with the decrease in the abundance of marine species and an increase of abundance of freshwater species (Thiel, 2011; Eick and Thiel, 2014).

Life cycle guilds

Beside their biogeographic affinity fishes can be categorized by their life cycle. Fish species in European estuaries were grouped into eight life cycle guilds by Thiel (2011):

1. **Marine straggler (M)**, marine species that are sporadically found in the lower estuarine sections with higher salinities: Atlantic mackerel (*Scomber scombrus*), red gurnard (*Chelidonichthys lucerna*).
2. **Marine estuarine-opportunists (O)**, marine species that are common and occur in high densities, primarily as juveniles in estuaries: Atlantic cod (*Gadus morhua*), Atlantic herring (*Clupea harengus* Linnaeus, 1758).
3. **Estuarine-marine (B)**, species that are able to complete their entire life cycle in both estuarine and marine environments: European anchovy (*Engraulis encrasicolus*) broadnosed pipefish (*Syngnathus typhle*).
4. **Estuarine (E)**, species that complete their entire life cycle within the estuary: common goby (*Pomatoschistus microps*), Nilsson's pipefish (*Syngnathus rostellatus*).
5. **Semi-anadromous (S)**, species that primarily spawn in the brackish water zone of estuaries but migrate back to marine waters for growing: three-spined stickleback (*Gasterosteus aculeatus*), meagre (*Argyrosomus regius*).
6. **Anadromous (A)**, species that spawn in freshwater but grow mainly in estuarine and marine environments: Atlantic salmon (*Salmo salar*), twaite shad (*Alosa fallax*).
7. **Catadromous (C)**, species that spawn in the sea but grow mainly in freshwater and estuarine environments: European eel (*Anguilla anguilla*), thinlip mullet (*Liza ramada*).
8. **Freshwater (F)**, freshwater species that occur in estuaries at lower salinities and in higher densities: zander (*Sander lucioperca*), northern pike (*Esox lucius*).

Overall, the diversity of life histories and recruitment mechanisms of taxa found in estuaries implies that these marine, estuarine, and diadromous fishes are adapted to the variable environments across these transitional habitats. As for other biota, salinity and temperature are the main factors influencing fish species distribution and community structure (Nissling and Westin, 1991; Thiel et al., 1995; Costa et al., 2002; Harrison and Whitfield, 2006). The longitudinal differences in species richness, evenness and biomass are caused by varying tolerance and preferences for salinity (Thiel et al., 1995).

Fish faunas and keystone fish species of the Elbe and Odra estuaries

According to Thiel (2011) about 80 fish species occur in the Elbe estuary, which makes it one of the more species rich estuaries in Europe. Smelt (*Osmerus eperlanus*) has been the most dominant fish species, since it occurs throughout the entire estuary with high density and biomass (e.g. Ehrenbaum, 1894; Lillelund, 1961; Möller, 1988; Thiel et al., 1995; Eick and Thiel, 2014). In the time span between 1984 and 2010 smelt displayed between 76 % and 96 % of the total fish density (Möller, 1988; Thiel et al., 1995; Thiel, 2001; Eick and Thiel, 2014). Since smelt belongs to the anadromous life cycle guild, anadromous fish species are the dominant life cycle guild (Eick and Thiel, 2014). Based on their frequencies of occurrence, ruffe and zander are the dominant freshwater fish species, while smelt and flounder are the dominant euryhaline fish species. Herring and gobiids of genus *Pomatoschistus* belong to the dominant marine fish species in the Elbe estuary (Thiel et al., 2025).

The fish fauna of the Odra estuary is structured across three main sections: the Pomeranian Bay, Greifswald Bodden and the Odra Lagoon. According to Thiel (2011) 50 fish species are counted in the Odra lagoon, whereas 61 fish species are estimated for the Greifswald Bodden. Smelt, ruffe and zander belong to the dominant fish species in the Odra lagoon (Lorenz, 2001). Due to its geographical location and hydrological regime, the Greifswald Bodden hosts species-rich fish fauna (Winkler 1989). Marine species from the Baltic Sea are permanently or temporarily present here, as well as freshwater fish species, that enter from the surrounding tributaries (Winkler 1989). In the 1960s, zander catches in the Bodden increased rapidly, which was linked to eutrophication (Winkler, 1989). As a result of the favourable feeding conditions, the zander in the Bodden exhibited the highest growth rates compared to stocks from other coastal waters (Winkler 1989). Although smelt occurred in the Greifswald Bodden, higher densities are more typical in the nutrient-rich lagoons of the Baltic (Haffe). Flounder occurs year- round in the Greifswald Bodden and provides relatively stable yields, whereas ruffe can occur in massive densities seasonally (Winkler, 1989). In the coastal waters of the Pomeranian Bay, species composition of the fish fauna was assessed using various sampling methods (Thiel et al., 2007). Using a mini-hopper-trawl, perch (61 %), zander (19 %), and flounder (15 %) were the dominant fish species. When sampled with an eel-trawl, flounder accounted for 67 % of the catch, followed by perch (12 %) and zander (13 %).

Keystone species are crucial for maintaining the structure and diversity of ecological communities and are therefore key targets in environmental protection efforts aimed at maximizing conservation outcomes (Paine, 1969; Mills et al., 1993). Across both estuaries, the keystone fish species zander, smelt, ruffe, and flounder were consistently present and were therefore selected for further feeding and growth analyses in this thesis.

1.3 European estuaries as feeding and nursery areas for fish

Importance of estuaries for fish

While estuaries constitute only to a comparatively small area (5.8 %), they are among the most important ecosystems in the coastal zone providing habitats for several plant and animal species (Barbier et al., 2011; Wetzel et al., 2014). They belong the most productive aquatic ecosystems and due to nutrient rich and diverse habitats they support a high fish production (Cronin, 1975; Thiel et al., 1995; Ducrotoy et al., 2019; Arevalo et al., 2023). Fishes are important elements in these aquatic communities. In estuarine food webs, keystone fish species are considered as suitable indicators for the assessment of ecosystem quality. They link lower with higher trophic levels such as piscivorous fish, birds and marine mammals or humans, when commercially fished. While estuaries are important ecosystems for a variety of biota worldwide, they also hold significant importance for humans. Historically, settlements developed along estuaries, benefitting from the location and resources. Cities that are located within reach of an estuary often host major ports serving as essential hub for shipping and trade. Both commercial and recreational fisheries can often be found, while tourism attracts visitors for various activities.

Estuaries are also highly important ecosystems for fish since they contain spawning grounds, feeding and nursery areas as well as migration routes for diadromous species. As migration route for fish estuaries are critical corridors for anadromous species (e. g. salmon *Salmo salar*) and catadromous species (European eel *Anguilla anguilla*) between freshwater and marine environments (Thiel, 2011). In addition to seasonal migrations, fishes also perform tidal migrations to forage in intertidal zones such as sandbanks, mudflats and tidal creeks (Costa et al., 2002). Several fish species including smelt (*Osmerus eperlanus*) or twaite shad (*Alosa fallax*) use estuaries as spawning and nursery area, since they find favourable conditions for their larval stages (Sepulveda et al., 1993; Thiel et al., 1995; Cabral et al., 2007; Guerreiro et al., 2021).

It is widely recognized that the annual recruitment variability of fish is primarily determined during the early life stages, particularly during the planktonic phase (Bolle et al., 2009; Houde, 2008; van der Veer et al., 2000; Guerreiro et al., 2021). The abundance and distribution of juvenile fish is strongly affected by quality and availability of the habitat, together with biotic (prey and predator abundance) and abiotic conditions (e. g. temperature, salinity, freshwater discharge) (Able et al., 2005; Bento et al., 2016; Martinho et al., 2007; Guerreiro et al., 2021). The high mortality rate of early life stages of fish (eggs and larvae), results from their limited swimming capabilities, which hinders them from escaping unfavourable environmental conditions, predation or areas with low food availability (Arevalo et al., 2023). In an intact estuary, the habitats generally offer direct physical protection for juveniles and larvae through seagrasses, wetlands, oyster reefs and/or shallow areas (Arevalo et al., 2023).

In the Elbe estuary, the shallow water of the freshwater region plays a critical role in fish production. The key areas, including the Mühlenberger Loch, Hahnhöfer Nebenelbe and Lühesander Süderelbe, have been identified as vital nursery grounds for fish larvae as well as juveniles of age group 0 (AG0) and AG1. Annual fish production (predominantly smelt) in these regions was up to 20 times higher, exceeding 200 kg per hectare, compared to other parts of the estuary (Thiel et al., 2001). The main spawning ground of smelt is located further upstream. The hatched larvae are transported by downstream drift into their shallow nurseries in the southern marginal areal (Ehrenbaum, 1894; Lillelund, 1961; Thiel, 2001).

In the Odra estuary, the Greifswald Bodden is a major spawning and nursery area for western Baltic spring-spawning herring (metapopulation), which was documented already in the 1930s (Biester, 1989; Polte et al., 2021). According to Polte et al. (2017), adult herring migrate from offshore pelagic habitats into inshore waters for spawning, where they attach their adhesive eggs predominantly to littoral substrates such as macrophytes. However, the reproduction and recruitment of herring in the region have faced significant challenges. Kanstinger et al. (2018) reported that the coverage of macrophytes in the Greifswald Bodden was reduced to 5 % compared to historical coverage of 90 % in this area. Seagrass declined due to light limitation and increased epiphytic algal growth driven by eutrophication. Despite the efforts to eutrophication reduction no increase of macrophyte coverage has been observed (Kanstinger et al., 2018). Furthermore, a reduction in herring larval survival has been linked to a phenological shift, potentially causing a temporal mismatch between the timing of herring spawning and the availability of adequate plankton prey.

European estuaries have four main functions for fish. Elliott and Hemmingway (2002) classified the function of 26 European estuaries based on the percentage of fish species on the total species number with following order: feeding area (75.8 %), nursery area (63.1 %) spawning area (24.3 %) and migration routes (8.9 %). This shows that European estuaries are especially important feeding and nursery areas for fish (Thiel, 2011).

Chapter 1 – Introduction

Estuarine food webs

In estuarine food webs, energy flows from the phytoplankton (primary production) and detritus through various trophic levels ultimately supporting higher trophic levels that consume a wide range of organisms. Generally, in aquatic food webs, primary production is consumed by zooplankton, which is then consumed by planktivorous fish, which are later consumed by predatory fish. In estuarine food webs, most of the primary production is not grazed alive, but being decomposed after death forming a detrital food web. A key characteristic of estuarine food webs is the importance of the benthic community, which inhabits filter feeders (clams, mussels), deposit feeders (worms, amphipods) and bottom feeding predators including other invertebrates (Day et al., 2012). The high availability of food resources ultimately results in large abundance and diversity of fish in estuaries (Beck et al., 2001; Trujillo and Thurman, 2013).

The coastal areas consist of diverse interconnected habitats, including pelagic waters, tidal creeks, seagrass meadows, mussel beds, coral reefs, intertidal flats, rocky shores and tidal wetlands, such as salt marshes and mangroves. While each habitat has its unique characteristic features, they are linked within a broader food web through fluxes of matter and energy (Hagy et al., 2012). Habitat connectivity is important for shaping trophic interactions in estuarine ecosystems, since an estuary can consist of various habitats. Heck et al. (2008) highlighted how shallow water seagrass meadows contribute to the adjacent ecosystems via trophic flows. For example, fish species that mainly live in mangroves often feed in seagrass meadows, generate a carbon transport from seagrass to mangrove (Hagy et al., 2012). The shallow nature of most estuaries allows benthic-pelagic coupling resulting in a nutrient transport between those two food webs (Hagy et al., 2012).

Feeding ecology of fishes in estuaries

Information on the food consumption of fish is central to understand aquatic food webs. There are biological controls such as prey availability and competition (both inter- and intraspecific) between fish and other predators. In bottom-up scenarios, the controlling factors are the lower trophic levels that affect the higher levels by resource restriction while in top-down control the lower trophic levels are affected by predation (Li et al., 2020).

According to Franco et al. (2008) the feeding mode of estuarine fish species can be grouped into eight categories according to their preferred food: Microbenthivores, who mainly feed on benthic, epibenthic, and hyperbenthic fauna, primarily targeting prey smaller than 1 cm. Macrobenthivores, who mainly feed on benthic, epibenthic, and hyperbenthic fauna, primarily target prey larger than 1 cm. Planktivores, who predominantly feed on zooplankton and occasionally on phytoplankton in the water column, mainly by filter feeding. Hyperbenthivores/zooplanktivores, who feed just above the benthos, predominantly on small mobile invertebrates and zooplankton. Hyperbenthivores/piscivores, who feed just above the benthos, predominantly prey on larger mobile invertebrates and fish. Detritivores, who feed on small organisms in or on the surface layer of the substratum, such as benthic algae (e.g. diatoms), including microfauna (Foraminifera and Flagellata), and smaller meiofauna. They ingest relatively large volumes of sand or mud by suction, digest the organic material and excrete the inorganic particles. Herbivores, who graze mainly on macroalgae and macrophytes and omnivores, who consume both plant and animal material due to feeding on macrophytes, periphyton, epifauna and filamentous algae.

In order to interpret the functioning of estuarine food webs, it is essential to consider prey availability not only in terms of abundance, but also in terms of detectability by fish. Experimental studies indicate that visual traits affecting prey selection include contrast with the background, size, movement, shape, colour, and unusual form features (Wootton, 1984; 1990; Elliott et al., 2002). The importance of visual predation on fish is reduced in estuaries, particularly in the maximum turbidity zone, due to limited visibility. Inhabiting these areas may therefore serve as a strategy for fish to avoid predation (Elliott et al., 2002).

The availability of suitable prey has a crucial impact on the larval fish survival. The size of available prey is one of the most critical factors in prey selection by fish larvae. This is closely tied to the larvae's mouth size and foraging abilities (Huang et al., 2021). During ontogenetic development, the mouth gape increases, which allows them to consume larger prey, alongside with improvements of swimming capabilities (Sabatés and Saiz, 2000). In early larval stages the small mouth limits the fish larvae to the smallest available zooplankton such as rotifers, nauplii, copepodites or cladocerans (Mehner and Heerkloss, 1994; Mehner and Thiel, 1999; Sabatés and Saiz, 2000; Thiel, 2001; Dickmann et al., 2007).

High densities, biomass and overall fish production rates of early life stages of smelt have been documented in the southern marginal areas of the Elbe estuary, which is close to the Hamburg port area in the freshwater region and concludes areas such as the Mühlenberger Loch, Hahnhöfer Nebenelbe and Lühesander Süderelbe. Here the production rates of smelt larvae and juvenile fish were substantially larger compared to the main channel and northern marginal area (Sepulveda et al., 1993; Thiel, 2001). These nursery areas were also characterized by high abundances of the copepod *Eurytemora affinis*, which has been identified as the dominant prey for fish in this region (Sepulveda et al., 1993; Bernát et al., 1994; Peitsch et al., 2000; Thiel et al., 1996; Thiel, 2001).

An example of how climate change can affect fish nurseries is seen in Baltic herring: in parts of the Odra estuary, larval herring suffer from starvation-related mortality due to a trophic mismatch. This mismatch results from the premature spawning of herring due to increased water temperatures, while zooplankton blooms occur later (Polte et al., 2021). After hatching, larvae rely on their yolk sac for nutrition. Once depleted, they depend on exogenous feeding, making the timely availability of suitable prey essential for survival (Seljeset et al., 2010; Fiksen and Jørgensen, 2011; Hernandez et al., 2018). Throughout the year, zooplankton production varies considerably in the Greifswald Bodden, rising sharply in March, peaking in June, and then declining thereafter.

Fish larvae that are unable to initiate feeding after yolk sac depletion rapidly approach a physiological threshold referred to as the "point of no return", typically reached within 3 to 5 days post-yolk resorption. Beyond this point, even if food becomes available, recovery is no longer possible and mortality is inevitable. This narrow window underscores the critical importance of precise timing between larval hatching and the availability of suitable prey in determining early survival success. That is why the southern marginal channels of the Elbe estuary are of such high importance as fish nurseries: they provide high densities of suitably sized prey organisms, mainly copepods (Sepulveda et al., 1993; Bernát et al., 1994; Peitsch et al., 2000; Thiel et al., 1996; Thiel, 2001), which are crucial for the growth and survival of larval smelt, ruffe, zander and flounder.

In general, the availability of prey is strongly linked with the growth, survival, and ultimately recruitment of fish during their early life history (Graeb et al., 2004). Nutrition, including the quality and quantity of food, plays a significant role in growth. Sufficient and balanced nutrition, especially protein, is critical for somatic growth. Poor imbalanced diets can lead to stunted growth or disease (Halver, 1954; Magnoni et al., 2019). The rate at which fish increase in length and weight over time is a direct indicator of how much suitable food is available, as faster growth typically reflects higher prey abundance and/or quality (Graeb et al., 2004; Cordoleani et al., 2022).

In conclusion, however, it should be emphasized that current and detailed studies on fish fauna structure, feeding ecology, and growth of keystone fish species in the Elbe and Odra estuaries are lacking but are urgently needed in order to improve our understanding of ecological processes in estuarine systems. This thesis includes multiple perspectives, ranging from population ecology and the overall species composition of the fish fauna to the feeding ecology and growth dynamics of specific keystone species, and ultimately to individual-level stress responses.

1.4 Objectives

Estuarine areas, such as those of the Elbe and Odra rivers, are influenced by a wide range of both natural and anthropogenic factors. They are characterized by the dynamic nature of environmental conditions (e.g., salinity, temperature, oxygen levels, turbidity), provide habitat for numerous plant and animal species and rank among the most productive aquatic ecosystems (Day et al., 2012). However, these regions are also highly impacted by overlapping human activities such as industry, shipping, fisheries, agriculture, nature conservation, sports, and tourism (Thiel, 2003). Overall, estuaries are among the most heavily anthropogenically altered habitats on Earth (Haedrich, 1983; Polgar et al., 1985; Whitfield, 1996; Cabral et al., 2001).

It is well known that human-induced factors in estuaries and other coastal marine systems can affect biodiversity, biomass, genetic structure and food webs (Gilarranz et al., 2016). In food webs, anthropogenic influences can alter their stability. Studies have also shown that anthropogenic influences are traceable in aquatic food webs, especially when the top predators are affected (Pauly et al., 1998; Pace et al., 1999; Myers and Worm, 2003). Fish play a critical role as top predators in estuarine food webs. Many planktivorous and benthivorous fish species act as key species in estuarine ecosystems, linking lower trophic levels with piscivorous fish and birds (Mehner and Thiel, 1999; Magath et al., 2016). Estuaries have been recognized as important fish habitats for more than a century, a role that has been repeatedly confirmed over time (Thiel, 2003). To gain deeper knowledge of the role of fishes in the food webs of temperate estuaries, in **chapter 2** the feeding ecology of four keystone fish species from two different temperate estuaries was analysed. With the Elbe estuary as mesotidal coastal plain estuary and the Odra estuary as microtidal bar-build estuary, we focused on two important estuarine types in central Europe. This chapter aims to (1) analyse and compare the overall food composition of keystone fish species in the two different estuaries, (2) identify spatial and seasonal trends in their food composition within each of the two estuaries and (3) estimate the dietary overlap between the keystone fish species.

For understanding the population dynamics of fish, the assessment of growth is an important tool (Nesslage and Pauly, 2022). In **chapter 3** the growth of four keystone fish species was investigated to identify regional differences between the estuaries and to compare current findings with previous results. These growth studies were used as an additional indicator of food availability, since increased food intake generally leads to faster growth.

Evaluation of indicator taxa has been established as simple measurement to be related to more complex environmental trends (Whitefield and Elliott, 2002). Although the ichthyofaunal composition in estuaries is highly reflecting the ever-changing environmental factors and life history cycles of various species, fish assemblages have often been used to illustrate changes of the conditions in estuarine ecosystems (Whitfield, 1996; Whitefield and Elliott, 2002), are regarded as suitable indicators that have been incorporated in European monitoring strategies (Water Framework Directive, Marine Strategy Framework Directive). In **chapter 4** the aim of our study was to analyse historical and present compositions of the ichthyofauna in the Elbe estuary using a dataset of comparable traditional fishing methods incorporating seasonal sampling since the 1980s (Möller, 1988; Thiel et al., 1995; Thiel and Potter, 2001; Thiel et al., 2003; Thiel, 2011; Eick and Thiel, 2014). Hereby we included: (1) assessing changes in life cycle guilds, (2) quantifying shifts in fish species densities, and (3) identifying long-term trends in environmental factors and their influence on fish population dynamics. In this context, the Elbe estuary serves as a representative case study for other temperate, mesotidal European estuaries, as it is one of the few systems in Europe with long-term quantitative data available for both fish communities and key environmental variables.

Although there is substantial knowledge of the abiotic and biotic changes occurring within estuarine systems, several spatio-temporal factors (temperature, salinity and dissolved oxygen) act as confounding variables, complicating the identification of the specific mechanisms driving observed declines in fish biomass (Koll et al., 2024). Molecular biological methods are increasingly being considered into biomonitoring and conservation biology. They offer insights into biological processes across multiple organizational levels and uncover interactions that were previously overlooked (Connon et al., 2018; Machuca-Sepúlveda et al., 2023, Koll et al. 2024). Multiple stressor interactions can lead to unforeseen synergistic or antagonistic effects, that can result in sublethal impact that contribute to long-term population declines across generations (Petitjean et al., 2019; Connon et al., 2018; Koll et al., 2024). In **chapter 5** we aimed to develop an unsupervised, network-based dimensionality reduction approach using real-world holobiont omics data derived from RNA and 16S rRNA libraries. The method was designed to accurately identify tissue-specific gene expression and shifts in bacterial community composition. By linking microbial dynamics to host immune responses and physiological measurements, we aimed to identify key environmental stressors and offer tools for the application of microbial biomarkers for the evaluation of fish health in estuarine ecosystems.

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Chapter 2 Inter- and intra-estuarine comparison of the feeding ecology of keystone fish species in the Elbe and Odra estuaries

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Abstract

Food webs in estuarine ecosystems serve as important biological indicators. The feeding ecology of four keystone fish species, pikeperch (*Sander lucioperca* L.), smelt (*Osmerus eperlanus* L.), ruffe (*Gymnocephalus cernua* L.) and flounder (*Platichthys flesus* L.), in the Elbe and Odra estuaries was analyzed using stomach content analyses. Important prey of pikeperch were fishes and mysids in both estuaries. Amphipods were especially important as prey for smelt in the Elbe estuary, whereas smelt caught in the Odra estuary mainly consumed mysids. Ruffe fed mainly on amphipods in the Elbe estuary, while annelids (lower section) and insect larvae (upper section) were the most important prey in the Odra estuary. Flounder favored copepods as prey in the Elbe estuary, while bivalves were preferred in the Odra estuary. Higher dietary overlaps were found in the Elbe estuary between smelt vs. ruffe, pikeperch vs. ruffe, and pikeperch vs. smelt. In the Elbe estuary, a shift in the diet composition of pikeperch, smelt, and ruffe was observed from 2021 to 2022 compared to food analyses from the 1990s. These shifts included an increased consumption of amphipods, while mysids and copepods had recently decreased in their diets. These changes indicate a restructuring of the food web, potentially linked to environmental changes, which highlights the sensitivity of estuarine ecosystems.

Keywords: estuaries; food webs; stomach content; fish

1. Introduction

Fishes are important elements in estuarine food webs, since they link the lower trophic levels with higher trophic levels such as other piscivorous fish, birds [1], marine mammals, or humans, when used commercially. Based on the relationships known to date, key fish species in the estuarine food web are considered suitable indicators for assessing the condition of estuarine ecosystems [2,3,4,5,6], as parameters such as their diet composition, nutritional intensity, growth rate, abundance, and spatial distribution depend on the availability of suitable habitats and food resources. Appropriate food resources availability at each specific stage of their life cycle is crucial for individual development and stock recruitment.

In estuarine ecosystems, multiple mechanisms shape the composition of food webs. First, prey availability and habitat differences play an important role, influenced by salinity gradients, substrate types, primary production, and detritus inputs [7,8,9]. Seasonal variability, including temperature changes and varying riverine freshwater discharge, affects the transport of prey organisms and triggers migration and reproductive cycles of prey species [10,11]. Interspecific competition also plays a significant role in structuring food webs [12]. Lastly, anthropogenic factors, such as habitat destruction, eutrophication, and pollution, can significantly alter prey composition in estuarine ecosystems [13,14].

However, the impacts of climate change can disrupt the timing of the availability of suitable food sources due to phenological mismatches. This occurs when the timing of phyto- and zooplankton blooms are misaligned with the hatching of fish larvae [15]. Other human-induced factors such as the loss of shallow water areas, hypoxic events, river dredging, increased turbidity, and contaminations, are believed to impair estuarine functions [5,16,17,18,19,20,21]. With recent shifts in the environmental factors, the fish abundance in the Elbe estuary, one of Europe's largest estuaries, was reduced by over 90 % [22], which included massive reductions in smelt, ruffe, and flounder. The reasons for this decline are multifactorial, including habitat destruction by river construction leading to increased turbidity, hypoxia events, changes in discharge dynamics, and a reduction in shallow water areas [22,23,24]. The Odra estuary is part of the Baltic Sea, where stocks of commercially important fish species such as cod (*Gadus morhua*) and herring (*Clupea harengus*) have been collapsing in recent years, indicating environmental stressors on the ecosystem, thus affecting the food web. The declines have been driven by a combination of climate change, nutrient loads, and overfishing [15,25,26,27]. Temperature is a key environmental factor influencing foraging behavior and prey–predator interactions, particularly in ectotherms. Climate change-induced temperature fluctuations affect metabolic rates, prompting animals to adjust their foraging preferences and efforts to meet their energy demands [28]. Recognizing this dynamic is crucial for understanding shifts in estuarine food webs under changing environmental conditions. Given the environmental challenges facing the Elbe and Odra estuaries, including climate-induced temperature shifts, habitat degradation, and anthropogenic pressures, this study provides insights into the feeding ecology of keystone fish species, ultimately influencing food web dynamics.

Smelt (*Osmerus eperlanus* L.), ruffe (*Gymnocephalus cernua* L.), flounder (*Platichthys flesus* L.), and pikeperch (*Sander lucioperca* L.) are among the keystone fish species in the Elbe estuary. These species occur throughout the entire longitudinal stretches of the Elbe estuary [22,29,30,31,32] and thus serve as key elements of the food web in the Elbe estuary. In the Oder estuary, Lorenz [33] revealed that smelt, ruffe, and pikeperch belonged to the dominant species in the Odra Lagoon, while according to Thiel et al. [34], flounder was among the dominant species in the coastal areas of the Pomeranian Bay. Therefore, these four species were collected for diet analyses.

To gain deeper knowledge of the feeding ecology of fishes in different temperate estuaries, this study was conducted on four keystone fish species from two temperate estuaries, the Odra as a microtidal estuary in the southern Baltic Sea and the Elbe as a mesotidal estuary in the south-eastern North Sea. This study aims to (1) analyze and compare their diet breadth and overall food composition in the two different estuaries, (2) identify spatial and seasonal trends in their food composition within each of the two estuaries, and (3) estimate the dietary overlap between pairs of the keystone fish species.

2. Materials and Methods

2.1. Study Area

The Elbe estuary is located in north-west Germany and extends from the weir at Geesthacht downstream to its mouth into the southern North Sea close to the city of Cuxhaven (Figure 2.1, left). It is strongly influenced by tides and is classified as a mesotidal coastal plain estuary with no marked halocline [32,35,36]. In the freshwater region or upper section (Mühlenberger Loch: ML, Twielenfleth: TF), the salinity is below 0.5 PSU, which stretches from the weir at Geesthacht (Ekm 586) to Stade (Ekm 654). In the brackish oligohaline area or middle section (Schwarztonnensand: ST), down to Brokdorf at Ekm 684, the salinity ranges between 0.5 and 5 PSU, and the brackish mesohaline area or lower section (Brunsbüttel: BB, Medemgrund: MG) continues downstream to the city of Cuxhaven at Ekm 725 [37].

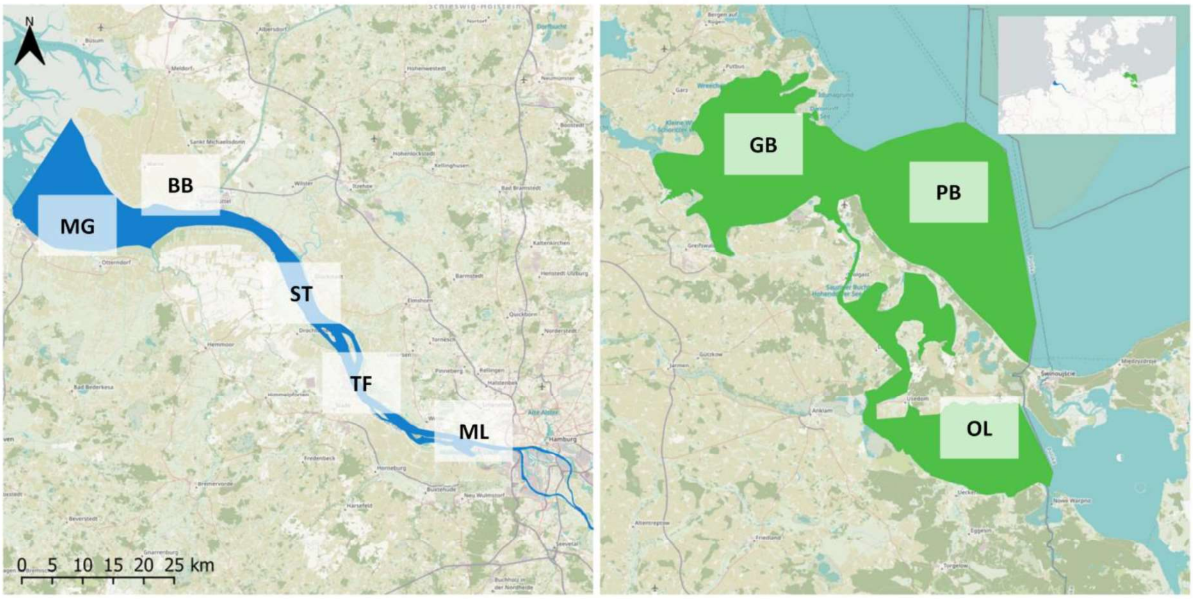

Figure 2.1. Elbe and Odra estuaries with the designated stations reflecting the longitudinal zonation. Elbe estuary (left): Medemgrund (MG) and Brunsbüttel (BB) as lower estuary, Schwarztonnensand (ST) as middle estuary, Twielenfleth (TF) and Mühlenberger Loch (ML) as upper estuary. Odra estuary (right): Pomeranian Bay (PB) as lower estuary, Greifswald Bodden (GB) as middle estuary and Odra lagoon (OL) as upper estuary (map sources: NextGIS, CartoDB).

The Odra estuary is located in north-east Germany and drains to the Baltic Sea (Figure 2.1, right) with very low influences from the tide with tidal oscillation below 20 cm [38]; it can therefore be classified as a microtidal estuary. We categorized the Odra estuary into three sections: the upper section (Odra

Lagoon: OL), with salinities ranging between 1 and 3 PSU, the middle section (Greifswald Bodden: GB), with a salinity range of 6–7 PSU, and the lower section (Pomeranian Bay: PB), with a salinity of 5–8 PSU [39,40].

2.2. Collection of Sample Material

We collected samples throughout the year, at least once per season: spring 2021 (May/June 2021), summer (August 2021), autumn (November 2021), winter (February/March 2021), and spring 2022 (May/June 2022). Fish samples were collected from commercial fishermen using the following catching techniques: stow net, fish traps, gill nets, trawl net. Fish were frozen at −20 °C immediately after catching or, at the latest, after landing, for preparation in the laboratory.

2.3. Analysis of Fish Diet

Diet analysis was carried out following Debus and Winkler [41] and Thiel [42]. The stomachs of the fishes were opened and the abundance, length, and weight of the prey species were determined. Through digestive processes, the biomass of the consumed prey organisms is reduced; hence, the actual consumed biomass at the moment of feeding cannot be determined by weighing. Therefore, the biomass was reconstructed by regression formulas following Debus and Winkler [41] as found in the literature (Table S1). Fragments were measured with a stereomicroscope Leica MZ 9.5 (±0.01 mm) combined with Leica Flexacam C1 (Leica LAS X software version 3.7.4.23463). From the fragment length, the total length and weight of the organism were calculated. Fragments or prey items that exceeded the maximum length of the stereomicroscope (>20 mm) were measured using millimeter paper. If no regression formula was available for an organism, standard weight was used. Whenever no regression formula and no standard weight was available, the available standard weight in the literature (Table S1) of the taxonomically and morphologically closest relative was used [41].

For species identification of consumed fish, characteristic bony structures (vertebral columns, singular vertebra, mandibular, pharyngeal teeth, otoliths) were used. Fish severely disintegrated by digestion were x-rayed (Faxitron MultiFocus) to portray identification features such as number of vertebrae or number of fin rays. The taxonomic identification of consumed prey objects was determined by characteristic features found in the literature: refs. [43,44,45,46,47,48,49,50,51,52] for fish and [53,54,55,56,57,58,59,60,61] for invertebrates. The identified prey organisms were assigned to taxonomic groups for later analyses (Gastropoda, Bivalvia, Annelida, Copepoda, Cladocera, Isopoda, Amphipoda, Thecostraca, Mysida, Decapoda, Insecta, Fish).

2.4. Calculation of Parameters in Feeding Ecology

For the characterization of the food composition, the following three parameters were calculated [62]: (I) Abundance (Ni): the abundance of prey types (i) to the number of all prey individuals, (II) frequency of occurrence (Fi): number of fish that ingested one prey type i in relation to the total number of fished which were analyzed, and (III) biomass (Bi): compares one prey individual’s weight per type i to the total weight of all prey individuals in percent.

$$I)\ N_i(\%) = \frac{n_i}{n} \times 100$$

$$II)\ F_i(\%) = \frac{N_i}{N} \times 100$$

$$III)\ B_i(\%) = \frac{RB_i}{RB} \times 100$$

n = Amount of prey items

i = Number of prey types i

N = Number of fishes with full stomachs

N_i = Abundance of prey type i

RB = Reconstructed prey biomass

RB_i = Proportion of prey type i in reconstructed prey biomass

Single index approaches for describing the diet can be biased [63], since they fail to capture the significance of each food component within the diet. The relative importance of prey taxa varies when only using single indices such Bi, Ni, or Fi alone. Small prey such as copepods occur in large numbers and are therefore represented with larger abundances (Ni) in the diet, while larger prey such as fish represent a larger biomass (Bi) although they occur in lower quantities. Using only the abundance of prey would overestimate the importance of the small prey ingested. To address this limitation, the Index of Relative Importance (IRI) proposed by George and Hadley [64] was calculated. The IRI combines multiple dietary metrics, providing a more comprehensive and accurate assessment of the importance of food components in the diet.

$$IRI\ (\%) = \frac{(B_i + N_i + F_i)}{\sum(B_i + N_i + F_i)} \times 100$$

The diet breadth (DB) indicates whether a species is a feeding generalist or specialist and was calculated following Levins [65]. The value of the DB can range from 1 to n. A high DB corresponds with higher diversity in the diet, and when DB is equal to 1 only one type of food was consumed. The Levins' DB not only provides information on the number of food components consumed but also about the distribution or evenness of the diet.

$$DB = \frac{1}{\sum_{i=1}^{n} pi^2}$$

pi = Proportion of prey item i

n = Total number of prey types

Dietary overlap (C_{xy}) was calculated with Schoener's [66] equation.

$$C_{xy} = \sum_{i=1}^{n} \min(p_{xi}; p_{xy})$$

pxi;pxy = Proportion of usage (IRI) for the resource i by the fish species x and y

n = Number of prey species

2.5. Statistical Analysis

Non-metric multidimensional scaling (NMDS) was conducted on untransformed diet composition data (IRI%) from each station using Bray–Curtis dissimilarities for the four keystone fish species. From the individual specimens, subsamples were created for each station: for smelt, 10 specimens per subsample, for pikeperch, 2 specimens per subsample, for ruffe, 5 specimens per subsample, and for flounder, 3 specimens per subsample. Analysis of similarities (ANOSIM) and later post hoc pairwise ANOSIM were conducted (999 permutations) to identify differences between groups (stations). For the pairwise comparisons, p-values were corrected via the Hochberg adjustment. Similarity percent (SIMPER) was conducted in order to determine the contribution of each prey taxon to the variation found in the NMDS ordination. The NMDS, ANOSIM, and SIMPER analyses were performed using the R-package 'vegan' (R version 4.1.2) [67].

3. Results

3.1. Food Composition and Diet Breadth

In the Elbe estuary, smelt (n = 643) with a size range from 75 to 226 mm, pikeperch (n = 84) with a size range from 76 to 190 mm, ruffe (n = 199) with a size range from 50 to 220 mm, and flounder (n = 287) with a size range from 24 to 383 mm were used for dietary analyses. In the Odra estuary, smelt (n = 102) from 76 to 210 mm, pikeperch (n = 121) from 105 to 219 mm, ruffe (n = 194) from 72 to 198 mm, and flounder (n = 118) from 84 to 339 mm were used. The highest diet breadth (DB) was estimated for flounder (6.8), followed by smelt (5.6), pikeperch (4.9), and ruffe (3.2). In the Odra, estuary flounder (6.5) was again the keystone species with the highest DB, followed by pikeperch (4.9), ruffe (4.2), and smelt (3.0). Pikeperch and flounder had similar DB in both estuaries, while for smelt the DB in the Elbe estuary was larger than in the Odra estuary. In contrast, the DB of ruffe was larger in the Odra estuary than in the Elbe estuary.

For pikeperch from the Elbe estuary (Table 2.1), fish as prey displayed 48.4 2% in total (index of relative importance = IRI, all following values referring the food composition are displayed as IRI). Most important prey species were smelt (34.31 %) and herring (6.27 %), while sprat (1.49 %), ruffe (2.99 %), and sand goby (1.44 %) were of lesser importance. The second most important prey taxon were mysids (23.65 %), mainly displayed by *Neomysis integer* (23.06 %). Amphipods displayed a moderately important prey taxon with 13.06 % (*Gammarus* spp.), together with decapods (14.88 %) (*Crangon crangon*: 5.13 % and *Palaemon longirostris*: 9.75 %).

Table 2.1: Food composition (% IRI) of keystone fish species in the Elbe estuary. Single prey components were categorized by their affiliation to higher groups (mesozooplankton, macrozoobenthos, zoobenthos, nekton). Higher groups in capitalized letters display the sums of the individual related prey taxa.

	Pikeperch	**Smelt**	**Ruffe**	**Flounder**
Diet breadth (B)	4.9	5.6	3.2	6.8
MESOZOOPLANKTON		**11.77**		**34.36**
COPEPODA		9.82		34.36
Eurytemora affinis		4.77		16.59
Calanoida indet.				17.77
Cyclopoida		0.20		
Copepoda-eggs		2.16		
Copepoda-Spermatophores		0.75		
Copepoda indet.		1.94		
CLADOCERA		1.95		
Daphnia galatea		0.78		
Daphnia longispina		0.56		
Daphnia spp.		0.50		
Bosmina longirostris		0.11		
Cladocera indet.		0.08		
MAKROZOOPLANKTON &	**51.56**	**70.60**	**91.62**	**34.58**
mainly HYPOZOOBENTHOS	23.65	19.40	23.11	17.01
Neomysis integer	23.06	15.82	21.19	17.01
Mesopodopsis slabberi	0.59	3.58	1.92	
AMPHIPODA	13.03	38.84	49.57	10.93
Gammarus spp.	13.03	35.61	48.43	10.93
Corophium volutator		3.23	1.14	
INVERTEBRATE EGGS		0.76		2.89
DECAPODA	14.88	11.60	18.94	3.75
Crangon crangon	5.13	6.21	13.15	3.75
Palaemon longirostris	9.75	4.67	4.49	
Eriocheir sinensis		0.72	1.30	
ENDOZOOBENTHOS &		**1.30**	**1.85**	**24.67**
BIVALVIA			0.20	0.59
Mya sp.				0.59
Pisidium spp.			0.20	
ISOPODA		0.46	0.17	8.00
Idotea balthica				6.56
Idotea emarginata		0.07	0.17	
Idotea granulosa		0.09		
Sphaeroma sp.				1.44
Isopoda indet.		0.30		
ANNELIDA		0.72		2.19
Hediste diversicolor		0.48		
Annelida indet.		0.24		
Tubificidae				2.19
INSECTA		0.12	1.48	13.89
Chaoborus sp.		0.07		
Chironomidae		0.05	0.63	13.89
Insecta indet.			0.85	
NEKTON (Fish)	**48.42**	**16.27**	**6.50**	**6.34**
Osmerus eperlanus	34.13	11.79	6.50	
Clupea harengus	6.27	3.27		
Sprattus sprattus	1.49			
Clupeidae	0.56			
Gymnocephalus cernua	2.99	0.52		
Sander lucioperca		0.05		
Pomatoschistus microps		0.06		
Pomatoschistus minutus	1.49	0.58		1.44
Merlangius merlangus				0.65
Platichthys flesus	1.49			
Fish Eggs				4.25

Table 2.2: Food composition (% IRI) of keystone fish species in the Odra estuary. Single prey components were categorized by their affiliation to higher groups (mesozooplankton, macrozoobenthos, zoobenthos, nekton). Higher groups in capitalized letters display the sums of the individual related prey taxa.

	Pikeperch	**Smelt**	**Ruffe**	**Flounder**
Diet breadth (B)	4.9	3.0	4.2	6.5
MESOZOOPLANKTON			**2.15**	
CLADOCERA			2.15	
Daphnia longispina			2.15	
MAKROZOOPLANKTON &	**41.70**	**69.50**	**24.80**	**15.31**
MYSIDA	15.27	53.03	2.64	2.46
Neomysis integer	15.27	53.03	2.64	2.46
AMPHIPODA	7.68	2.07	19.5	4.54
Gammarus spp.	7.68	2.07	11.95	4.54
Corophium volutator			7.55	
INVERTEBRATE EGGS		0.49		
DECAPODA	18.75	13.91	2.66	8.31
Crangon crangon	18.09	13.91	1.30	4.02
Palaemon longirostris	0.66			
Eriocheir sinensis			1.36	
Carcinus maenas				4.29
ENDOZOOBENTHOS &	**3.40**	**7.44**	**72.82**	**84.72**
GASTROPODA	0.43	0.36	2.32	11.71
Potamopyrgus jenkinsi	0.43			
Hydrobiidae		0.36	2.32	11.71
BIVALVIA	0.74	0.40	2.70	48.03
Mya arenaria	0.74			
Mya sp.				30.91
Mytilus edulis				7.95
Cerastoderma edule				8.85
Mysella bidentata		0.40		
Limecola balthica				0.32
Bivalvia indet.			2.70	
THECOSTRACA				0.53
ISOPODA		1.49	1.56	3.77
Idotea balthica			0.40	3.77
Idotea granulosa			1.16	
Idotea chelipes		1.49		
ANNELIDA	1.75	5.19	25.50	13.58
Hediste diversicolor	1.75	5.19	25.50	13.58
INSECTA	0.48		40.74	7.10
Chironomidae	0.48		39.85	7.10
Insecta indet.			0.89	
NEKTON (Fish)	**54.89**	**23.07**	**0.28**	
Osmerus eperlanus			0.28	
Clupea harengus	1.75			
Gymnocephalus cernua	0.58			
Pomatoschistus minutus		14.30		
Pomatoschistus spp.	17.15	8.77		
Neogobius melanostomus	0.97			
Alburnus alburnus	0.76			
Rutilus rutilus	33.68			

Amphipods (38.84 %) were the most important prey organisms for smelt, which was mainly displayed by *Gammarus* spp. (35.61 %). The second most important food taxon for smelt were other fishes (16.26 %), which consisted mainly of other smelt (11.79 %, cannibalism) and herring *Clupea harengus* (3.27 %). The third most important prey taxon were mysids, displayed mainly by *Neomysis integer* (15.82 %). Copepods were also an important prey of smelt (9.82 %), which mainly consisted of *Eurytemora affinis*. In smaller amounts, other benthic invertebrates such as the annelid *Hediste diversicolor* and isopods (*Idotea emarginata*, *Idotea granulosa*) with 0.46 % were consumed by smelt.

For ruffe, amphipods (49.57 %) were the most important prey organisms (*Gammarus* spp.: 48.43%). These were followed by mysids (23.11 %), consisting mainly of *Neomysis integer* (21.19%), decapods with 18.94 % (*Crangon crangon*: 13.15 %, *Palaemon longirostris*: 4.49 %, *Eriocheir sinensis*: 1.30 %), and fish with 6.50 % (smelt). Insect larvae (Chironomidae: 0.63%, insect larvae indet. 0.85%) were of minor importance for ruffe from the Elbe estuary.

For flounder, copepods (34.35 %) were the most important prey taxon, which were followed by mysids with 17.01% (*Neomysis integer*), insect larvae with 13.89 % (Chironomidae), amphipods with 10.93 % (*Gammarus* spp.), isopods 8.00 % (*Idotea balthica*: 6.56 %, *Sphaeroma* sp.: 1.44 %), fish 4.90 % (fish-eggs: 4.25%, *Merlangius merlangus*: 0.65 %). Bivalves were of minor importance 0.59% (*Mya* sp.) for flounder from the Elbe estuary and gastropods were not consumed at all.

In the Odra estuary (Table 2.2), pikeperch fed mostly on other fishes, with a proportion of 54.89% consisting mainly of *Rutilus rutilus* (33.68 %) and *Pomatoschistus* spp. (17.15 %). The second most important prey taxon was displayed by decapods (18.75 %), which consisted almost exclusively of *Crangon crangon* (18.09 %). Mysids with 15.27 % were exclusively displayed by *Neomysis integer*. Amphipods, displayed by *Gammarus* spp. (7.68 %), were of minor importance in the diet of pikeperch from Odra estuary.

3.2. Spatio-Temporal Variability of Food Composition Within the Estuaries

3.2.1. Pikeperch

Polygons representing the stations in the NMDS ordination showed overlap for the samples from all stations in the diet composition of pikeperch (Figure 2.2). The pairwise ANOSIM indicated that significant differences existed solely between stations ST (Elbe) and GB (Odra) (R = 0.721, p = 0.028). In the Elbe estuary, the proportion of piscivorous diet of pikeperch was largest in the lower and upper sections (MG: 75.9 %, ML: 59.9 %), while in the middle section (ST: 12.5 %) the amount of fish was smallest (Figure 2.3). In the middle section, the importance of mysids (47.6 %) and amphipods (30.8 %) was substantially greater compared to the other regions in the Elbe estuary. In the Odra estuary, the proportion of fish in the diet of pikeperch was largest in the middle section (GB: 81.8 %), compared to the upper section (OL: 59.7 %) and lower section (PB: 35.7 %). In contrast, the proportion of decapods in the diet of pikeperch was higher in the lower section (35.2 %) of the Odra estuary, while in the upper (4.1 %) and middle section (2.1 %), the proportions of decapods were substantially smaller. In both estuaries, the proportion of fish in the diet of pikeperch was higher during the summer compared to the autumn samples (Figure 3). In autumn, the proportion of decapods and mysids increased in both estuaries, whereby in the Odra estuary the general diversity of ingested prey taxa increased.

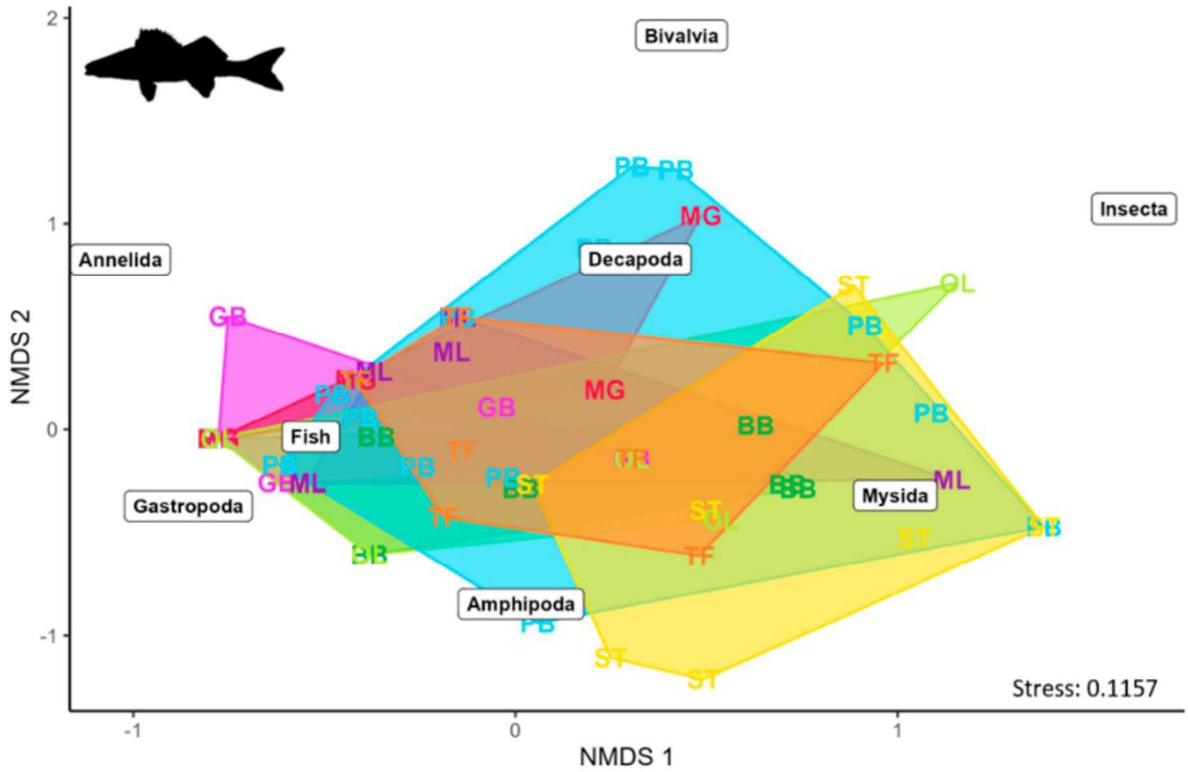

Figure 2.2. NMDS ordination on food composition, calculated from the index of relative importance (% IRI) on Bray–Curtis dissimilarity matrix (top): ANOSIM (R = 0.086, p = 0.0301) of juvenile pikeperch (*Sander lucioperca*) from Elbe (n = 84) and Odra (n = 121) estuaries. Elbe estuary (left): Medemgrund (MG), Brunsbüttel (BB), Schwarztonnensand (ST), Twielenfleth (TF) and Mühlenberger Loch (ML). Odra estuary (right): Pomeranian Bay (PB), Greifswald Bodden (GB) and Odra lagoon (OL).

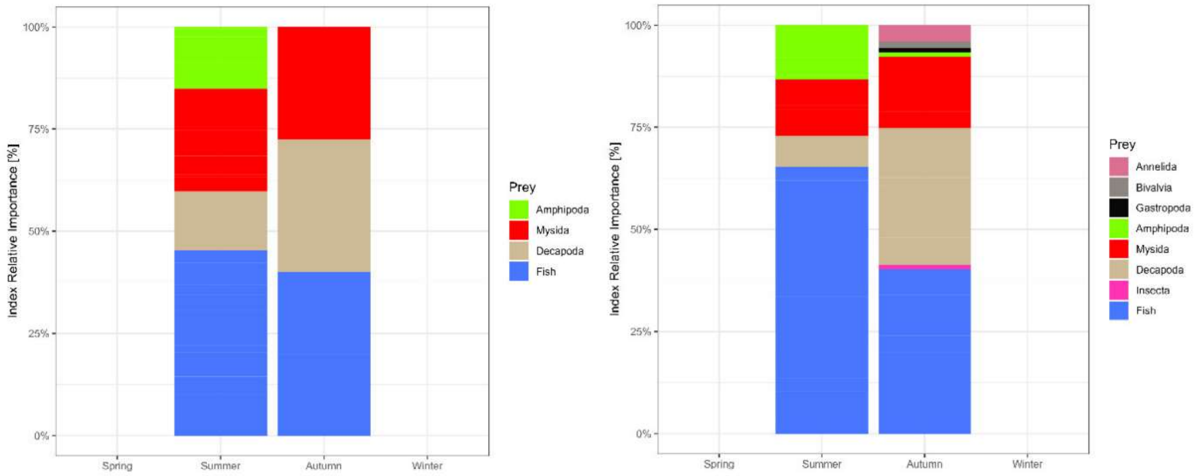

Figure 2.3. Seasonal variation in the prey composition of juvenile pikeperch (*Sander lucioperca*). (Left) Elbe estuary (summer n = 76, autumn n = 8), (right) Odra estuary (summer n = 75, autumn n = 46).

3.2.2. Smelt

The NMDS ordination of the diet composition of smelt revealed that most stations in the ordination space overlap, except for the PB station which is orientated separately from the other stations (Figure 2.4). Significant dissimilarities in food composition were found at the stations in between the two estuaries: BB vs. PB (R = 0.659, p = 0.012), ML vs. PB (R = 0.733, p = 0.012), ST vs. PB (R = 0.703, p = 0.012), and TF vs. PB (R = 0.684, p = 0.012). In the Elbe estuary, copepods were absent in the diet of smelt caught at the lower section (MG). The proportion of copepods in the diet of smelt gradually increased in the upstream direction: BB (1.5 %), ST (7.3 %), TF (15.3%), ML (29.2 %). Amphipods reached the highest proportion of the IRI in the middle section at ST (47.5 %). The importance of mysids in the diet of smelt was largest at the lower sections (MG: 25. 3%, BB 26.7 %). Isopods (MG: 0.6 %, BB: 1.6 %) and annelids (MG: 1.0 %, BB: 1.3 %) were exclusively found in the diet of smelt from the lower sections. Cladocerans were exclusively found in samples from the middle and upper sections of the Elbe estuary. In the Odra estuary smelt could only obtained from the lower section (PB), so the overall description of the food composition corresponds to “3.1 Food composition and diet breadth”. In the Elbe estuary, seasonal differences in the food composition of smelt were found. In particular, during spring (45.5 %) and autumn (41.5 %), the percentages of amphipods were higher in the diet of smelt. In spring and autumn samples (Figure 2.5), amphipods were the dominant prey, while mysids dominated in summer samples with 40.0 %, and copepods were the most important prey in winter (43.5 %). In the Odra estuary, samples from summer and autumn were available. Notably, the amount of fish in the diet of smelt was larger in summer (49.7 %) compared to autumn (14.7 %). On the contrary, mysids were less important as a food resource during summer and had increased later in the autumn season.

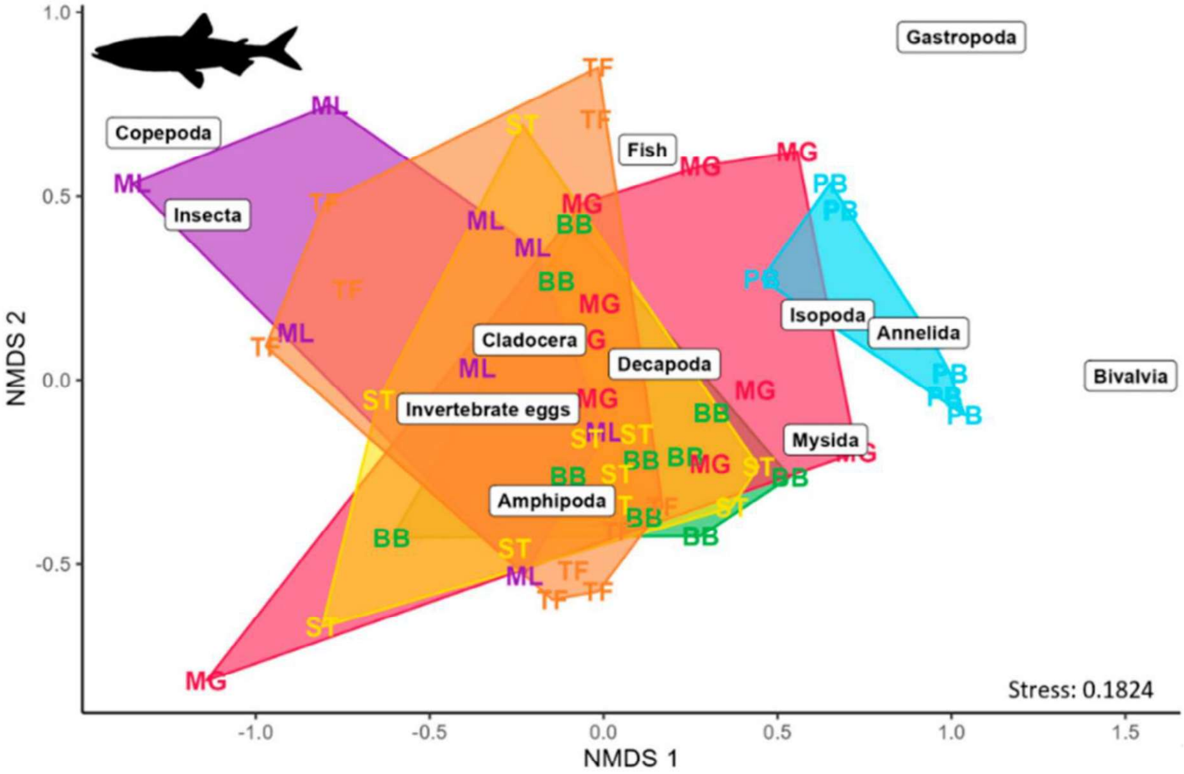

Figure 2.4. NMDS ordination on food composition, calculated from index of relative importance (% IRI) on Bray–Curtis dissimilarity matrix (top): ANOSIM (R = 0.231, p < 0.001) of smelt (*Osmerus eperlanus*) from Elbe (n = 660) and Odra (n = 103)

estuaries. Elbe estuary (left): Medemgrund (MG), Brunsbüttel (BB), Schwarztonnensand (ST), Twielenfleth (TF), and Mühlenberger Loch (ML). Odra estuary (right): Pomeranian Bay (PB), Greifswald Bodden (GB), and Odra lagoon (OL).

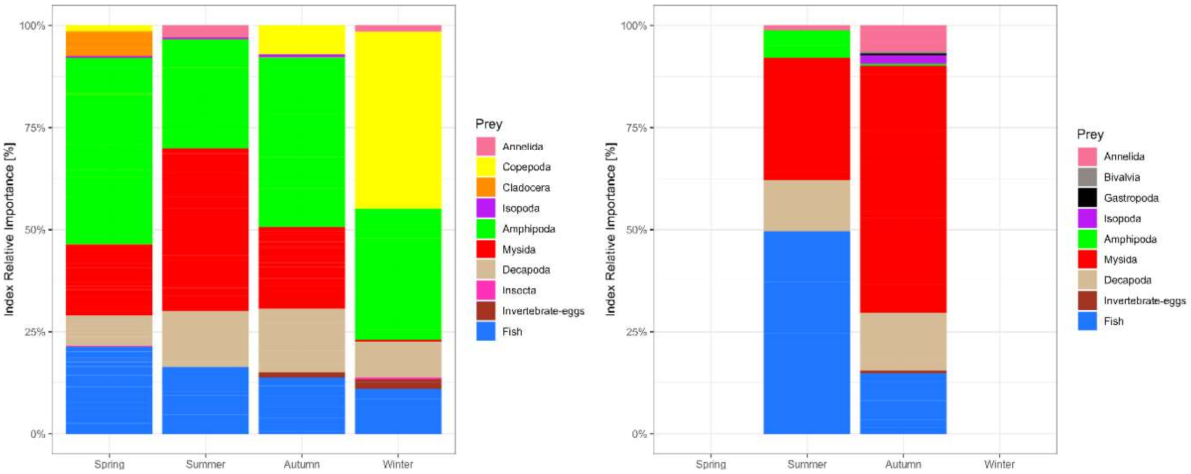

Figure 2.5. Seasonal variation in the prey composition of smelt (*Osmerus eperlanus*). (Left) Elbe estuary (spring n = 183, summer n = 100, autumn n = 235, winter n = 125), (right) Odra estuary (summer n = 43, autumn n = 59).

3.2.3. Ruffe

The NMDS ordination shows that samples from the Elbe estuary were grouped in clusters, while being separated from samples of the Odra estuary. Samples from the Odra estuary were less overlapping compared to samples from the Elbe estuary (Figure 2.6). Pairwise ANOSIM shows significant dissimilarities between all stations between Elbe and Odra estuaries: BB vs. GB (R = 0.781, p = 0.036), BB vs. PB (R = 0.913, p = 0.016), BB vs. OL (R = 0.755, p = 0.028), ST vs. GB (R = 0.848, p = 0.016), TF vs. GB (R = 0.702, p = 0.049), ML vs. GB (R = 0.624, p = 0.016) ML vs. PB (R = 0.926, p = 0.028), ML vs. OL (R = 0.659, p = 0.049), ST vs. PB (R = 0.947, p = 0.016), TF vs. PB (0.856, p = 0.049), ST vs. OL (R = 0.834, p = 0.016), TF vs. OL (R = 0.605, p = 0.049). Within the Elbe estuary, ML vs. ST (R = 0.305, p = 0.049), was the only comparison that showed significant dissimilarities, while within the Odra estuary, PB vs. OL (R = 0.784, p = 0.016), and GB vs. PB (R = 0.663, p = 0.036) were significantly different. For ruffe in the Elbe estuary, amphipods were the most important prey organisms at all stations, while in the upstream regions, the IRI of amphipods was slightly elevated (ML: 63.1 %, TF: 55.8 %). Fish in the diet of ruffe increased alongside the salinity gradient, with highest IRI in the upstream regions (ML: 15.1 %, TF: 10.5 %). Insect larvae were only found in small amounts in the middle (2.4 %) and upper (2.0 %) region of the Elbe estuary in the diet of ruffe.

Decapods were important prey organisms from the middle to the lower sections. In the Odra estuary, insect larvae were the most important food resource of ruffe. Here, the IRI was highest in the upper region (OL: 66.9 %) and declined alongside the salinity gradient, with 39.9 % in the middle region (GB) and 4.5 % in the lower region (PB). In contrast, the most important prey in the lower region were annelids, which decreased along the salinity gradient when moving upstream: middle region (21.4%), upper region (no annelids found in the diet). Amphipods were the most important prey organisms during all four seasons (Figure 2.7), although in slightly lower amounts during summer (46.8 %) and autumn (45.0 %) compared to spring (54.5 %) and winter (53.9 %). Seasonal differences in the diet of ruffe were most pronounced in the winter season, while in all other season, mysids were an important part of the diet; during winter, no mysids were found. Ruffe consumed other fish throughout the year, except during winter. In the Odra estuary, the amount of insect larvae was particularly lower in autumn (32.4 %), while in the same season, the proportion of annelids (37.4 %) was highest. Amphipods were consumed throughout all seasons, and the proportion was largest in winter (34.9 %) and smallest during summer (6.7 %).

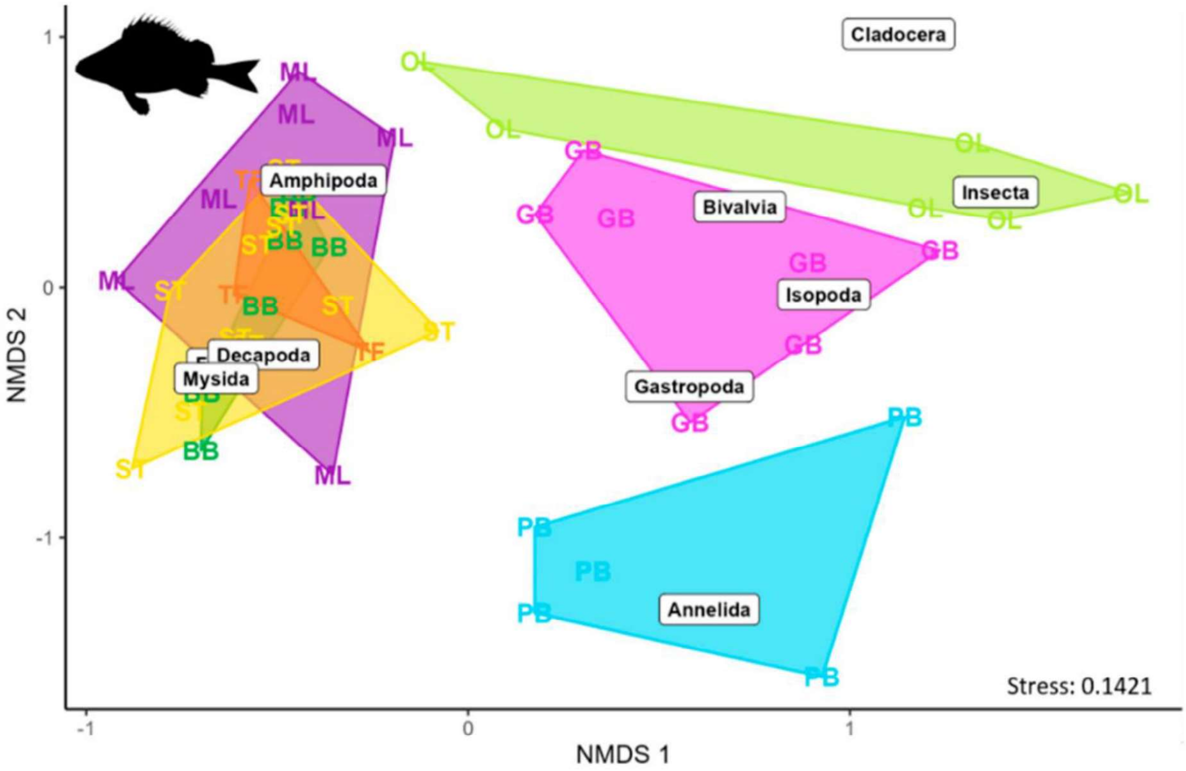

Figure 2.6. NMDS ordination on food composition, calculated from index of relative importance (% IRI) on Bray–Curtis dissimilarity matrix (top): ANOSIM (R = 0.549, p < 0.001) of ruffe (*Gymnocephalus cernua*) from Elbe (n = 198) and Odra (n = 194) estuaries. Elbe estuary: Medemgrund (MG), Brunsbüttel (BB), Schwarztonnensand (ST), Twielenfleth (TF), and Mühlenberger Loch (ML). Odra estuary (right): Pomeranian Bay (PB), Greifswald Bodden (GB), and Odra lagoon (OL).

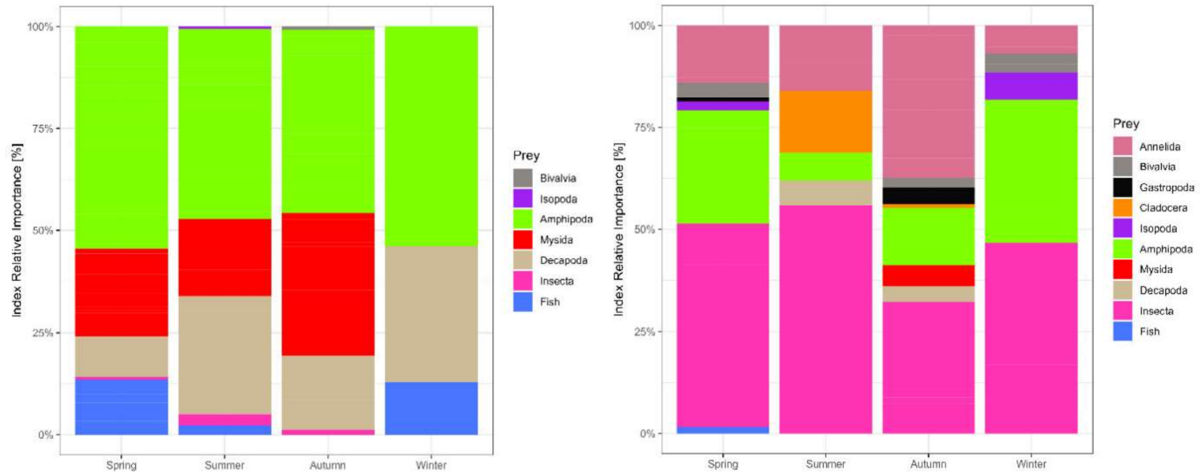

Figure 2.7. Seasonal variation in the prey composition of ruffe (Gymnocephalus cernua). (Left) Elbe estuary (spring n = 64, summer n = 71, autumn n = 52, winter n = 13), (right) Odra estuary (spring n = 53, summer n = 26, autumn n = 81, winter n = 34).

3.2.4. Flounder

Food composition reflected by the NMDS ordination showed that most samples of flounder were partly overlapping while some were not. In particular, samples from the Odra estuary (PB and GB) were separated in the ordination space (Figure 2.8). Considerable differences were found between stations in the Elbe and Odra estuaries: BB vs. PB (R = 0.958, p = 0.018), MG vs. GB (R = 0.448, p = 0.011), ML vs. GB (R = 0.633, p = 0.016), MG vs. PB (R = 0.876, p = 0.011), and ML vs. PB (R = 0.954, p = 0.032). Within the Elbe estuary, MG vs. ML (R = 0.598, p = 0.018) and MG vs. TF (R = 0.641, p = 0.032) revealed significant dissimilarities, while in the Odra estuary dissimilarities of GB vs. PB (R = 0.405, p = 0.018) were significant. For flounder in the Elbe, the proportion of copepods in the diet was smallest in the lower regions of the estuary (MG: 4.8 %, BB: 3.0 %). In the upstream direction, the proportion of copepods successively increased: ST (37.5 %), TF (50. %), and ML (67.3 %). Insects in the diet were only found in the upstream region (TF: 38.3%, ML 25.0%), while mysids were found in the diet of flounder caught at the middle and lower regions of the estuary. In the lower region at BB, the mysids displayed a proportion of 48.5 %. Isopods were found in small amounts in all regions, except for the most upstream station, ML. In the Odra estuary, in the lower region, bivalves were the dominant food resource (PB: 64.3 %). In the middle section, the food composition was not as dominated by one food resource compared to the lower region (Figure 2.9). In the upper regions of the Odra estuary (OL), no flounder were captured, hence no feeding data were available. At GB, bivalves (25.5 %) were the most important prey, followed by annelids (23.7 %) and insects (17.0 %). The proportion of mysids in both regions was comparatively small, with 3.9 % in the lower region and 0.5 % in the middle region. Isopods were of larger importance for flounder from the middle section (7.6 %) compared to the lower section

(1.0 %). The diet composition of flounder from the Elbe estuary displayed high seasonal variability (Figure 2.9). During spring (47.1 %) and winter (48.0 %), copepods were the most important prey organisms. The proportion of mysids was substantially larger in spring (22.8 %), summer (42.6 %), and autumn (21.6 %) than in winter (3.6 %). Insect larvae were predominantly found in flounders from autumn, with an IRI of 53.7 %, compared to summer (12.5 %), and with no insects during spring and winter. Amphipods, in contrast, were only found in Elbe flounder caught in spring (15.7 %) and winter (17.4 %). In the Odra estuary, high seasonal variability was found in the diet of flounder. Bivalves were predominantly found in flounders from summer (63.0 %) and autumn (54.8 %). Isopods were an important food resource during winter (26.8 %) compared to the other seasons (spring: 3.7 %, summer: 3.8 %, autumn: 1.1 %). Annelids were especially important prey organisms during spring (45.6 %) and winter (31.7 %), while decapods were particularly important during autumn (18.2 %).

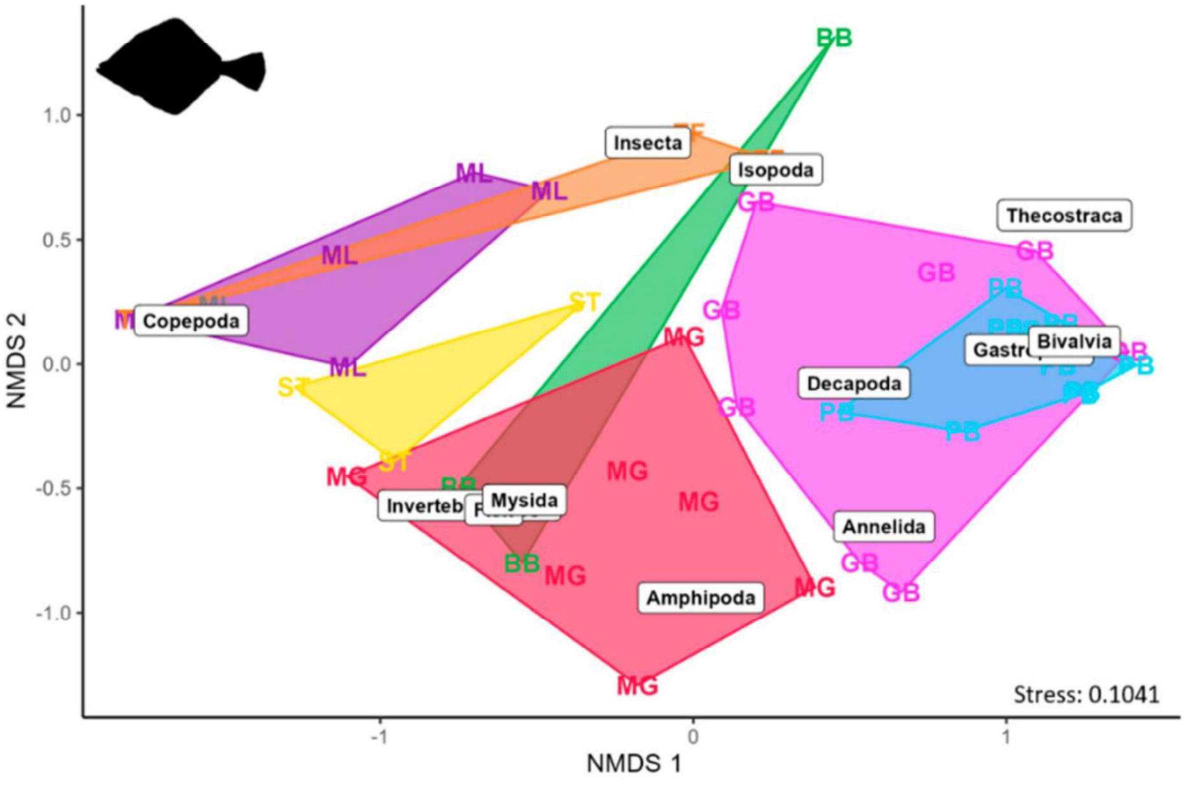

Figure 2.8. NMDS ordination on food composition, calculated from index of relative importance (% IRI) on Bray–Curtis dissimilarity matrix (top): ANOSIM (R = 0.646, p < 0.001) of flounder (*Platichthys flesus*) from Elbe (n = 281) and Odra (n = 94) estuaries. Elbe estuary (left): Medemgrund (MG), Brunsbüttel (BB), Schwarztonnensand (ST), Twielenfleth (TF), and Mühlenberger Loch (ML). Odra estuary (right): Pomeranian Bay (PB), Greifswald Bodden (GB), and Odra lagoon (OL).

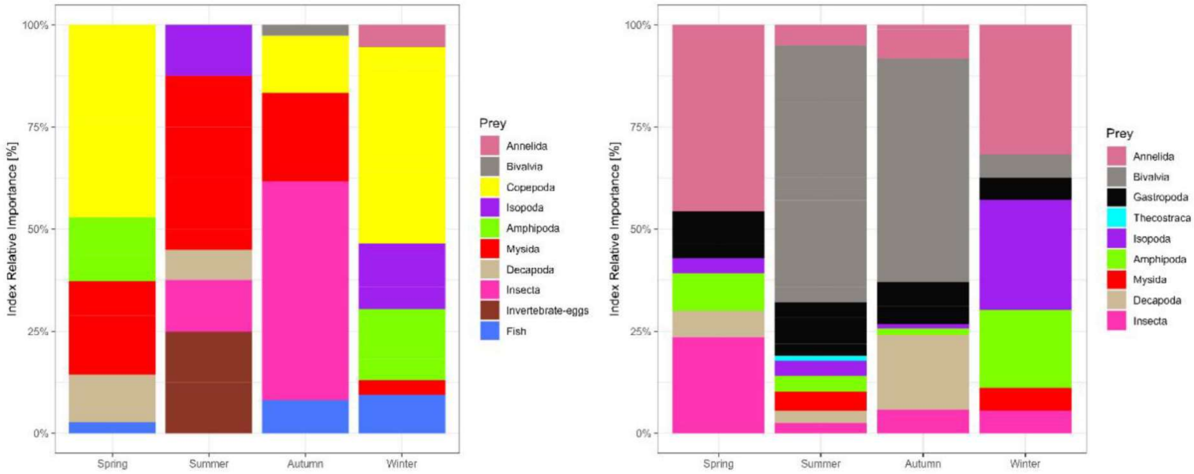

Figure 2.9. Seasonal variation in the prey composition of flounder (*Platichthys flesus*). (Left) Elbe estuary (spring n = 81, summer n = 62, autumn n = 81, winter n = 63), (right) Odra estuary (spring n = 16, summer n = 73, autumn n = 26, winter n = 3).

3.3. Dietary Overlap in the Elbe and Odra Estuaries

Generally, the dietary overlap between the keystone species was substantially larger in the Elbe estuary than in the Odra estuary (Figure 2.10). This was true for all species combinations except for ruffe/flounder (Elbe Cxy = 0.325, Odra Cxy = 0.319) and pikeperch/smelt (Elbe Cxy = 0.553, Odra Cxy = 0.472), in which the dietary overlap was only slightly larger in the Elbe compared to the Odra. A high similarity in diet composition was found between ruffe and smelt (Cxy = 0.703), which was the highest calculated dietary overlap for the keystone fish species compared in pairs in the Elbe estuary. In the Oder estuary, the dietary overlap between the keystone fish species, especially ruffe and smelt, was substantially smaller (Cxy = 0.119).

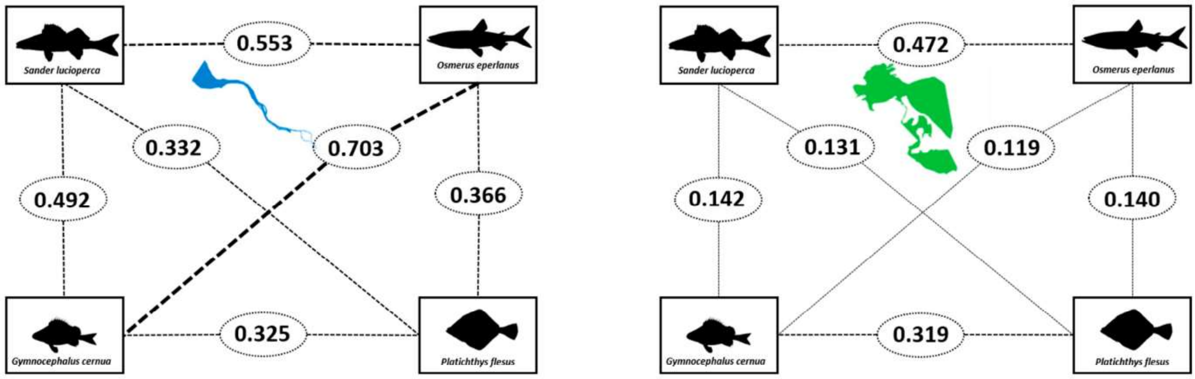

Figure 2.10. Dietary overlap (Schoener's index) of keystone fish species from Elbe and Odra estuaries. Higher dietary overlap is indicated by thicker lines.

4. Discussion

4.1. Spatial Feeding Variability

Notably, pikeperch consumed less fish and more invertebrates (amphipods, mysids, decapods) at station ST, compared to the other stations. The reduced consumption of fish at ST may in turn be a consequence of the lower food availability of fish (smelt), especially in the middle region of the Elbe estuary. The preference of pikeperch for smelt as a prey species of pikeperch in the Elbe estuary is consistent with findings of Kafemann and Thiel [68]. In a previous study, we found that smelt densities were much lower in the middle region (ST: 6905 individuals per 1 Mio m^3) compared to the upper region (ML: 32,307 individuals per 1 Mio m^3) and the lower region (MG: 30,307 individuals per 1 Mio m^3) [22]. Koll et al. [69] showed that conditions and food supply were unfavorable for juvenile pikeperch in the middle estuary. At the station ST, the condition factor (Fultons' body condition factor K) was lower compared to the other stations, and pikeperch expressed starvation on a molecular level (transcriptomes). This also reflects the findings of van Densen [70], who described a large growth difference between piscivorous and non-piscivorous juvenile pikeperch. Juvenile pikeperch appear to be flexible predators that specialize in different prey taxa, preferably fish, depending on the food resources of the ecosystem they inhabit. Although a piscivore diet is important for juvenile pikeperch regarding their survival and growth, the lack of preferred prey organisms can lead to juvenile pikeperch feeding predominantly on less nutritious taxa such as mysids (*Neomysis integer*) and amphipods (*Gammarus* spp.).

The food composition of smelt in the Elbe and Odra estuaries differed primarily in terms of the proportions of fish, mysids, and gammarids. The smelt in the Elbe estuary consumed fewer fish (Elbe estuary: IRI = 16.26 %, Odra estuary: IRI = 23.07 %), fewer mysids (Elbe estuary: IRI = 19.4 %, Odra estuary: IRI = 53.03 %), and more gammarids (Elbe estuary: IRI = 35.61 %, Odra estuary: 2.07 %) than in the Odra estuary. In smelt, a gradual change in diet along the longitudinal gradient of the Elbe estuary was observed, with copepods becoming more important upstream, while the importance of mysids declined. *Eurytemora affinis* displays the most abundant copepod species in the Elbe estuary and was found in the highest densities in the freshwater zone in southern marginal areas close to ML [71]. Temperature has a larger affect on the reproductive parameters of *Eurytemora affinis* compared to salinity, as population densities were described to decrease drastically when water temperature exceeds 20° C [72,73]. The primary production in the maximum turbidity zone (MTZ) of the Elbe estuary was low, with a year-round low quality of particulate organic matter (POM) [74,75]. As a result, there is limited food available for *Eurytemora affinis* in this region. The high turbidity results in rather unfavorable living conditions for phototrophic organisms, where phagotrophy provides crucial advantages for mixotrophic flagellates [76], leading to trophic lengthening [75]. Enriched δ15N in juvenile smelt was found in the MTZ but not in adults, which means that the adults avoid areas with inappropriate environmental conditions and limited food availability [75]. In the diet of smelt in the Eru Bay (Gulf of Finland), amphipods and mysids were the most common prey, while fish in the diet were important prey organisms, particularly for larger smelt from August to October [77]. In the Gulf of Riga, juvenile smelt strongly selected the cladoceran *Bosmina longispina* as prey. It was concluded that the fish may switch to consume other prey organisms when the preferred food is limited [78].

Fish in the diet of ruffe increased with lower salinities, with the highest IRI observed in the upstream regions. Insect larvae were only found in small amounts in the diet of ruffe in the middle and upper regions of the Elbe estuary. Decapods were important prey organisms from the middle to the lower sections but not in the upper estuary. In the Odra estuary, insect larvae were the most important food resource for ruffe. The IRI was highest in the upper region and declined with higher salinities in the middle region (GB). In the lower region (PB) of the Odra estuary, insect larvae were of minor importance in the diet of ruffe. The most important prey in the lower region were annelids, which decreased in the upstream regions. This is due to the fact that insect larvae and the annelid *Hediste diversicolor* have a certain salinity requirement [79]. In non-tidal estuaries such as the Couronian Lagoon, adult ruffe prey on juvenile smelt and perch, alongside meso- and macrozooplankton. Similarly, in the Darß-Zingst Bodden, ruffe also consume nine-spined sticklebacks (*Pungitius pungitius*) [80]. In other freshwater habitats (e.g., Lake Võrtsjärv, Lake Aydat), chironomid larvae and pupae were the dominant prey of ruffe [81,82].

In the Elbe estuary, copepods became more prominent in the diet of flounder in the middle and upper regions, likely due to the preference of *Eurytemora affinis* for the freshwater zone in the southern marginal areas [71]. As flounders are migratory species [83], they move through these regions where copepods are more abundant. In the Odra estuary, bivalves (*Mya arenaria*) were the dominant prey in the lower region (PB), where the Odra Bank is located. This shoal, characterized by sandy sediments, supports a benthic community dominated by *Mya arenaria* [84]. A proportion of benthic food components (approx. 50%) has also been determined previously for the Elbe estuary [85] and has also been found in other estuaries, such as the Schlei [86], the Westerschelde [87], the Humber estuary [88], the Severn estuary [89], and the Tagus estuary [90]. This shows that the feeding preference of flounder from the Elbe estuary for copepods and zoobenthic taxa aligns with findings from other European estuaries. In the Odra estuary, no copepods were consumed, while zoobenthic taxa accounted for a higher proportion of the IRI (84.72%), indicating a stronger preference for benthic prey in this system. Flounder in the northern Baltic Sea (Åland archipelago) also showed strong seasonal variations in the diet of flounder [91]. In the south western Baltic Sea, the main prey of flounder was *Limecola balthica* [92].

Differences in food composition between the two estuaries can be attributed to the prey availability and generalist feeding behavior of the keystone species, since they exploit a wide range of prey resources. Consequently, fluctuations in prey availability across different locations result in distinct food compositions. The Elbe and Odra estuaries are ecologically and hydrologically quite different: The Elbe is a mesotidal estuary with strong tidal influences and high flow velocities, which shape its ecological conditions. In contrast, the Odra estuary is microtidal, with minimal tidal influence. Its lagoon-like structure, rather than a prominent channel, results in larger water residence time in the OL [93]. This creates conditions that support different prey species, whereas in the Elbe estuary, tidal currents and daily mixing result in fluctuations in salinity. In the Odra estuary, for example, bivalves are prevalent on sandbanks, while insects reproduce in large numbers under the oligohaline conditions of the OL.

4.2. Temporal Feeding Dynamics

Seasonal variations in the food composition for pikeperch were more pronounced in the Odra estuary. In the Elbe estuary, food compositions between summer and autumn were quite similar. In the Odra estuary, pikeperch consumed less fish and a larger variety of prey taxa, including benthic invertebrates such as annelids, gastropods, and isopods during autumn. Additionally, the proportion of decapods was substantially larger in autumn. This suggests that the preferred fish species were less available in autumn than in summer. Studies conducted in the Kiel Canal (NOK) by Kafemann and Thiel [68] found that pikeperch experienced reduced growth because the NOK lacked a sufficiently high supply of fish as their preferred food, including herring [94] and smelt in particular [70,95]. In the 2021–2022 period, pikeperch consumed gammarids at nearly all stations (except MG at the river mouth), while gammarids were not found at all in the food of pikeperch in the Elbe estuary during the 1992–1993 period [70].

In the Elbe estuary, seasonal differences in the food composition of smelt were most pronounced in winter, with copepods being the dominant prey. This suggests a significantly higher availability of copepods during late winter. In contrast, in the Odra estuary, fish were the most important prey during summer but were replaced by mysids in autumn. Smelt fed predominantly on gobies from the genus *Pomatoschistus*, which occur most abundantly during late summer months in the Baltic. Pikeperch [96] showed that the biomass of *Pomatoschistus minutus* in the Baltic peaked in August/September and decreased substantially later in October. As the availability of *Pomatoschistus* sp. as a food resource decreased in autumn, smelt shifted their main diet to mysids. Compared to the findings from Thiel [42], a lower proportion of preyed upon smelt were found in the food of smelt (cannibalism) in the Elbe estuary in the 2021–2022 period than in the 1991–1993 period (IRI = 11.79 % vs. IRI = 23.74 %).

Overall, fish made up a substantially lower proportion of the smelt's diet composition in 2021–2022 than in 1991–1993 (IRI = 16.27 % vs. IRI = 26.03 %). Similarly to the pikeperch, the proportion of the consumed gammarids in 2021–2022 (IRI = 35.61 %) was notably higher than in 1991–1993 (IRI = 3.08 %), while the proportion of the *Neomysis integer* was similarly high during both periods (IRI 2021–2022: 15.82 %, IRI 1992/1993: 14.31 %). The proportion of mesozooplankton, especially that of calanoid copepods of the species *Eurytemora affinis*, was also highly variable. The number of copepods in the diet of smelt in the Elbe estuary from 1991 to 1993 was IRI = 11.78 % [42], while the percentage during 2021–2022 was significantly smaller (IRI = 4.77 %). Ladiges [97] described smelt from the Elbe estuary as planktonic feeders, which are highly dependent on *Eurytemora affinis* but periodically consume benthic invertebrates. It was further stated that between November and March, benthic organisms were exclusively consumed, mainly displayed by the genus Gammarus, and it was concluded that smelt undergo a hunger period in winter, during which they shift to less desirable prey organisms such as gammarids. In the samples from 2021 to 2022, gammarids were a major food component all year round, indicating a limitation in the preferred food resources for smelt in the Elbe estuary. The primary production in the maximum turbidity zone (MTZ) of the Elbe estuary is low, with year-round low quality of particulate organic matter (POM) [74,75], providing limited food resources for grazing copepods. Hauten [75] found enriched δ15N in juvenile smelt in the MTZ but not in adults and concluded that the adults avoid areas with inappropriate environmental conditions and limited food availability. The consumption of indigestible objects such as small pieces of wood, stones, plant matter, and sand was observed most frequently during winter in our samples, which was also described by Ladiges [97] during starvation periods.

The seasonal diet variation in ruffe was not as pronounced as in the other keystone species in both estuaries. Across all four seasons, a similar proportion of amphipods was consumed, while in winter, ruffe did not feed on mysids. In the Odra estuary, especially during autumn, ruffe fed on annelids. In summer, ruffe from the Odra estuary fed on cladocerans. Long-term studies in the Baltic Sea have shown that temperature and salinity influence the abundance of cladocerans, and that densities were highest during summer [98]. By comparing the recently analyzed diet of ruffe with previous studies from the Elbe estuary, further indications of a change in food availability were found: Thiel [42] determined very different food compositions for ruffe in this area for the 1991–1993 period. In that period, ruffe from the Elbe estuary consumed a relatively high proportion of copepods of the species *Eurytemora affinis* (IRI = 26.21 %), while in our study, no copepods were found in the diet of ruffe from 2021 to 2022. In the 1990s, gammarids were consumed by ruffe, with an IRI = 7.55%. In the samples from 2021 to 2022, samples were estimated to contain a substantially larger proportion of gammarids (IRI: 48.43 %). Amphipods have a sturdy cuticula, which is formed by chitin and calcium (ash, i.e., amount of inorganic non-combustible material), which makes them of lower nutritional value compared to other prey organisms with no cuticula (e.g., fish) or those with less sturdy cuticula (e.g., mysids). The study by Thiel [42] did not report fish in the diet of ruffe, which changed in the samples from 2021 to 2022, where fish (smelt) were ingested by ruffe (IRI = 6.5 %).

The diet composition of flounder was the most variable among the keystone fish species, likely due to its generalist feeding behavior [99]. In the Elbe estuary, copepods were a major dietary component during spring and winter. In the Odra estuary, annelids made up a larger proportion of the diet in spring and winter, while bivalves were more dominant in summer and autumn. This seasonal difference may be due to a preference for annelids, as they lack a hard shell, making them easier to digest and a more favorable prey option. Although the prey composition of flounder changed between these two periods, the differences were not as pronounced as for the other species. In both periods, 1991–1993 [42] and 2021–2022, flounder consumed copepods in high proportions in the Elbe estuary (IRI 1991–1993: 41.29%, IRI 2021–2022: 34.36 %). The zoobenthos species changed from an IRI of 14.4 % (1991–1993) to an IRI of 24.7 % (2021–2022). Both groups (copepods, zoobenthic taxa) together accounted for more than 50 % of the IRI of flounder during both periods in the Elbe estuary.

Seasonal shifts in salinity, temperature, and freshwater inflow across seasons can induce the reproductive cycles of both predators and prey. The temperature affects the metabolic rate of fish, influencing their feeding requirements [100]. In contrast to the spatial salinity gradient in estuaries, seasonal changes in river discharge affect the salinity gradient and therefore the distribution of biota. The changes in river discharge also affect plankton dynamics [101], as higher discharge increases the downstream transport of planktonic organisms. The salinity gradient in estuaries plays a crucial role in shaping the composition and distribution of biota in estuaries [21,30,42,102]. Generalist feeders opportunistically exploit a wide range of prey resources. As a result, fluctuations in prey availability lead to temporal dietary shifts, with opportunistic predators adapting to seasonally abundant or accessible food sources [103]. The combination of seasonal shifts in temperature, salinity, and river discharge can trigger spawning and recruitment dynamics, which shape the fish composition [21,30,32,104]. In the Elbe estuary, the spawning migration of smelt in late winter is induced by the increased river discharge [105]. By comparing the food composition of keystone fish species from the Elbe estuary with historical data, a shift from a predominantly plankton-based food web to a more benthos-based food web is observed. This transition could be an indication of possible responses to shifting environmental conditions, since the Elbe estuary has a long history of anthropogenic interventions.

Hypoxia has been shown to affect feeding behavior in several fish species [106,107,108], primarily reducing foraging intensities and metabolic rates, which leads to growth slowing. The oxygen situation in the Elbe estuary has been documented by several authors within the last 40 years

[21,29,32,109,110]. Especially in the Hamburg Port region (upper estuary), severe hypoxic events have occurred. Although the oxygen situation improved compared to the 1980s [24], the situation has declined in recent years leading to recurring hypoxic events. Especially during years with lower river runoff, areas with low oxygen concentrations have extended upstream to Bunthäuser Spitze at Ekm 609. These hypoxic events during summer months could have impaired the feeding activities of the keystone fish species in the Elbe estuary. Accordingly, changes in feeding strategies can occur under different turbidity conditions, with visual predators being more affected by turbidity than fish that feed on benthic macroinvertebrates [111]. For visual predators, the turbidity of the water columns plays a crucial role in their predation success. Increased turbidity could also be an explanation for the dietary shift towards extended feeding on amphipods. Generally, pikeperch and smelt feed on more pelagic organisms, particularly smelt, which primarily consume calanoid copepods, and pikeperch, which primarily feed on other fish. However, as turbidity increased [24], visual predation might have been impaired, causing a shift towards more benthic foraging. We suspect these shifts in food composition were induced by the availability of prey, with accessibility changing foraging behavior as a result of changed environmental conditions.

4.3. Dietary Overlap

Sharing an abundant food source is not necessarily direct evidence of competition, but it can be caused by high abundances of certain prey organisms, leading to food selection [112]. Schoener's values that are higher than 0.6 or lower than 0.4 are considered to be of ecological relevance [113,114,115,116,117]. When comparing the dietary overlaps of the key species in the Elbe estuary from 2021 to 2022 to historical data from the 1990s [42], the general overlaps in the 1990s were smaller overall, especially due to lower abundances of amphipods in the diet. The high dietary overlap (0.703) of smelt and ruffe in the Elbe estuary is an indication for relevant competition. The findings from the Elbe estuary indicate either a competition for amphipods due to a lack of other suitable prey taxa or a general high availability of this prey taxon.

5. Conclusions

The differences in the food composition of keystone fish species from Elbe and Odra estuaries were probably primarily influenced by the availability of prey organisms. The preference for a piscivorous diet was clear in pikeperch from both estuaries, although the availability of fish as prey fluctuated within the estuaries. In the Elbe estuary, smelt fed extensively on amphipods, while in the Odra estuary, amphipods played a subordinate role and mysids were the preferred food objects. Ruffe in the Elbe estuary preferred mainly amphipods as prey, while in the Odra estuary, insect larvae and annelids were the preferred food, with preferences changing along the salinity gradient. Flounder had the most diverse diet, especially in the Elbe estuary, with preferences for copepods, mysids, and insect larvae, while in the Odra estuary, bivalves, annelids, and gastropods were preferred. Seasonal differences in food composition were driven by fluctuations in prey availability, leading to temporal dietary shifts as opportunistic predators adapted to seasonally abundant or accessible food sources. This effect was most pronounced in flounder, the most generalist feeders, as reflected by the highest diet breadth (DB) among the keystone species. Compared to investigations from the 1990s, the food composition in the Elbe estuary has shifted, with a notable increase in amphipods as a dietary component in pikeperch, smelt, and ruffe. Therefore, the food composition of keystone fish species in the Elbe estuary showed indications of shifting from a plankton-based (copepods, mysids) food web to a more benthos (amphipods)-orientated system. Together with trophic elongation of the food chain, these shifts could have been induced by changed environmental factors, which may further have ecological impacts on phytoplankton and zooplankton that ultimately affect higher trophic levels, promoting altered foraging behavior. The increased dietary overlap between species in the Elbe estuary indicate an increased competition for existing food resources, which could be the result of limited food availability in the Elbe estuary.

6. References

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8. Institutional Review Board Statement

Our Fish were caught by commercial fishermen, who applied the standards of the German Animal Welfare Act (§4 TierSchG) for killing the Fish. From these catches, we took our samples (dead specimens), therefore our research did not involve live animals.

9. Author Contributions

J.T.: conceptualization, investigation, field work, data collection and processing, statistical analyses, writing of the manuscript, design of figures, project administration. S.S., S.A.-M. and J.D.: investigation, data collection and processing, review and editing. E.H.: field work, investigation, review and editing. R.K.: field work, investigation, review and editing. C.M. and A.F.: conceptualization, funding acquisition, review and editing. R.T.: funding acquisition, conceptualization, supervision, review and editing, project administration. All authors have read and agreed to the published version of the manuscript.

Chapter 3 Growth patterns of key stones fish species in temperate estuarine ecosystems – a comparison between the Elbe and Odra estuaries

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Abstract

The Elbe and Odra estuaries are essential feeding grounds, nursery and growth areas for several fish species. With climate change and human activities (e. g. channel management, wastewater sewage and nutrient inputs) both estuaries have faced several anthropogenic stressors in the past and in the present. Growth rates in fish often reflect the availability of suitable prey, with abundant food resources and adequate nutrition typically promoting faster growth. This study aimed to analyse the growth patterns of four keystone fish species to identify regional differences of two temperate estuarine ecosystems (Elbe and Odra estuaries), and to compare current findings with past studies. Length and age of representative samples of four keystone fish species, zander (*Sander lucioperca*), smelt (*Osmerus eperlanus*), ruffe (*Gymnocephalus cernua*) and flounder (*Platichthys flesus*) were analysed, as these species occur in high numbers throughout both estuaries and play a key role in the respective aquatic food webs. In addition to length measurements and age determinations, the size ranges of individual age groups and the parameters of the von Bertalanffy growth function were calculated for growth assessment. The asymptotic lengths of smelt, ruffe and flounder were larger in the Elbe compared to the Odra estuary, while the growth coefficients were smaller in the Elbe. When comparing the growth of zander, smelt and ruffe from the Elbe estuary with previous studies, it becomes noticeable that the growth in the present study was inferior to values reported in past studies. In particular, ruffe showed a lower asymptotic maximum length ($L\infty$), and smelt had smaller age-specific mean lengths compared to earlier studies. The growth of juvenile zander was even distinguishable within the Elbe estuary, with reduced growth in the maximum turbidity zone in the middle estuary. The slower growth observed at these sites appears to be associated with a reduced intake of fish prey. Juvenile zander at these locations relied more heavily on invertebrates such as amphipods, mysids and decapods compared to individuals from other stations. In the Odra estuary, flounder displayed a smaller asymptotic length compared to other studies from the Baltic. Still, flounder had a higher growth coefficient in contrast to studies from the Pomeranian Bay and Gulf of Gdańsk. Our findings indicate that changes in environmental conditions and food availability, particularly in the Elbe estuary, are affecting fish growth patterns, highlighting the importance of ongoing monitoring to identify the underlying causes.

1. Introduction

Estuaries belong to the most heavily anthropogenically altered habitats on earth (Haedrich, 1983; Polgar et al., 1985; Whitfield, 1996; Cabral et al., 2001). These areas experience a high degree of overlap from human activities, such as industry, shipping, fisheries, agriculture, nature conservation, sports and tourism (Thiel, 2003). Given to the yet known relationships, key fish species are considered suitable indicators for assessing the condition of estuarine ecosystems (e.g. Costa and Elliott, 1991; Deegan and Garritt, 1997; Hughes et al., 1998; Whitfield and Elliott, 2002; Borja et al., 2004; Harrison and Whitfield, 2006; Breine et al., 2007; Courrat et al., 2009). The Elbe and Odra estuaries are influenced by a broad range of both natural and anthropogenic factors. They are characterized by dynamics of natural environmental variables, such as salinity, temperature, oxygen and turbidity. Both estuaries provide habitats for various plants and animal species and are among the most productive aquatic ecosystems.

Estuaries have been recognized to harbour important habitats for fish for over a century, with their significance in this function reaffirmed (Thiel, 2003). They serve primarily as feeding and nursery areas, while they also play a role as spawning areas and migration routes (Elliott and Hemmingway, 2002;

Thiel, 2011). Anthropogenic stressors such as the loss of shallow-water areas, maintenance dredging, hydromorphological alterations, reduced river runoff, increased turbidity, cooling water extraction, hypoxia, and pollution are suspected to disturb the function of estuaries as feeding and nursery habitats (e.g. Gibson, 1994; Cabral et al., 2001; Whitfield and Elliott, 2002; Able, 2005; Gilliers et al., 2006; Thiel, 2011; Theilen et al., 2025a).

Metrics such as general species abundance, spatial distribution, diet composition, feeding intensity and growth can be evaluated in order to assess the ecosystem function and quality. Information on growth is an important element for understanding the population dynamics of fish (Nesslage and Pauly, 2022): The relationship between length and age reveals when an individual might become susceptible to fishing mortality and if the fish had chances for reproduction when data on maturity is available. In fisheries, often the largest and most fecund individuals within a population are harvested (Darimont et al., 2009; Uusi-Heikkilä et al., 2015). The quantitative relationships between length and age of fish are commonly used to convert length data into age data for stock-assessment models. This relationship can be quantified by using the standard von Bertalanffy curve (von Bertalanffy, 1938).

The growth of fish can act as an indicator for food availability, as greater food consumption generally results in faster growth (Goodrich and Clark, 2023). Naturally, fish experience periods of reduced food intake during their life. During these periods, energy reserves are redirected away from growth in order to maintain vital body functions (Sumpter et al., 1991). Poor habitat conditions such as low dissolved oxygen levels have been shown to reduce growth rates (Campbell and Rice, 2014; Cottingham et al., 2014). However, water temperature still is the physio-chemical factor that affects fish growth the most (Searcy et al., 2007).

The aim of this study was to analyse the growth of four keystone fish species in the Elbe and Odra estuaries to identify regional differences between and within the estuaries to compare current findings with previous studies. These growth studies were carried out in order to act as an additional indicator for food availability.

2. Material and Methods

2.1 Sample collection

Fish samples were collected throughout the year and at least once per season, by commercial fisheries using following the catching techniques: stow net, fish traps, gill nets and trawl net (Theilen et al., 2025b). The samples were frozen immediately after catching or, at the latest, after landing. Samples originated from five stations in the Elbe estuary: Medemgrund (MG), Brunsbüttel (BB), Schwarztonnensand (ST), Twielenfleth (TF), Mühlenberger Loch (ML) and from three stations in the Odra estuary Pomeranian Bay (PB), Greifswald Bodden (GB), Odra lagoon (OL).

2.2 Data acquisition

The age of fish can be determined by using hard structures (Fig. 3.1), as visible annual ring structures (annuli) form due to seasonal differences in growth (Campana, 2001). Most fishes have an increased growth during the warmer months of the year due to higher temperatures (metabolic rates) and better food availability, whereas growth slows down or even comes to a complete halt during the colder months (Schneider, 2001). This seasonal variation in growth intensity causes summer rings to be wider than winter rings. Depending on the fish species, different hard structures were used for age determination. For validation of the age estimation, multiple hard structures were evaluated per species:

- European smelt (*Osmerus eperlanus*): otoliths, scales
- Flounder (*Platichthys flesus*): otoliths
- Ruffe (*Gymnocephalus cernua*): otoliths, operculum, scales
- Zander (*Sander lucioperca*): total length measurements indicated that all individuals belonged to age group 0

These four species occur throughout the Elbe and Odra estuaries in high numbers and thus serve as key elements of the aquatic food web (Möller, 1988; Thiel et al., 1995; Thiel et al., 2003; Lorenz, 2001; Thiel et al., 2007; Theilen et al., 2025a; Theilen et al., 2025b)

The age determination was conducted using a Leica MZ 9.5 stereomicroscope. At least ten scales per specimen were analysed, since scales can regrow after being lost due to injuries and regrown scales do not reflect the age correctly. Therefore, only scales below the dorsal fin on the left body side were used for age readings. The scales were cleaned after extraction and were placed between two microscope slides. Two sagitta otoliths (when possible) and one operculum were used per specimen. Otoliths were cleaned and dried after removal, and opercula were placed in boiling water in order to remove skin and soft tissue from the bone. Age determination was performed independently by two readers. When both readers came to the same result, the reading was considered valid for further analyses.

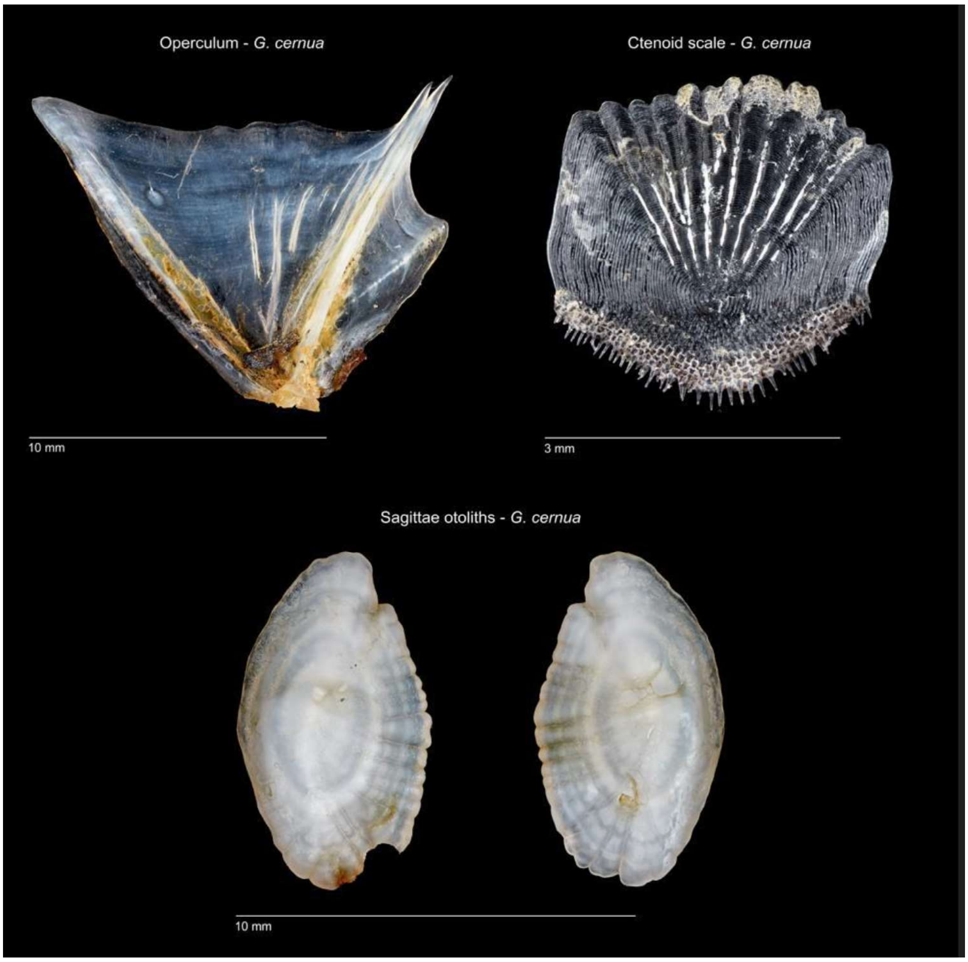

Figure 3.1: Hard structures used for age determination in ruffe (*Gymnocephalus cernua*). Top left: operculum; top right: scale; bottom: sagitta otoliths. Photos: T. Weddehage in Storz (2023).

2.3 Growth parameters

As a basis for comparing the growth of zander between the estuaries, the total lengths per age group 0 were visualized as boxplots. Since there were no zander analysed from other age groups than 0, no Bertalanffy curve was calculated. For smelt, ruffe and flounder, multiple age groups were analysed from both estuaries. These comprehensive data allowed the application of the von Bertalanffy growth function (VBGF) to compare length growth between the two estuaries the VBGF is given by following equation:

$$L_t = L_\infty \cdot (1 - e^{-K(t - t0)})$$

L_t = total length at time t
t = time (age) in years
L_∞= total length at an age of ∞ (=asymptotic length)
K = specific growth coefficient
t_0 = time where total length is equal to zero

Fulton's condition factor K_c (Fulton, 1902) was calculated as the indicator for the fish health. The Fulton conditions factor decreases with increasing body length of a specimen and can be used to compare fish populations living under different conditions (Ighwela et al., 2011):

$$K_c = \frac{100 \times W}{L^3}$$

K_c = Fulton's condition factor
W = body weight in g
L = total length in cm

2.4 Statistical tests

For the comparison of juvenile zander sizes as well as condition factors between stations (for all keystone fish species), Kruskal-Wallis H-test were conducted, followed by a post-hoc Dunn's test with Bonferroni correction. Mann-Whitney U-test was used to compare the size ranges of individual age groups between the two estuaries.

3. Results

3.1 Growth

3.1.1 Zander

In the Elbe estuary, the total length of age group 0 (AG0) zander differed significantly between stations (Kruskal-Wallis H-test, $p < 2e-16$). Significant differences were observed between all stations except ML vs. MG as well as ST vs. TF (Dunn's test, $p = 0.528$ for both comparisons). Individuals from MG and ML had significantly larger total lengths compared to the other stations, while the lowest total lengths were recorded at ST and TF (Fig. 3.2). Significant differences in total length were found between the following station combinations (the larger one named first): MG vs. BB (Dunn's test, $p = 0.001$), ML vs. BB (Dunn's test, $p = 0.002$), BB vs. ST (Dunn's test, $p = 0.0002$), BB vs. TF (Dunn's test, $p = 0.001$), MG vs. ST (Dunn's test, $p = 1.73e-15$), Twielenfleth and Medemgrund (Dunn's test, $p = 1.86e-8$), ML vs. ST (Dunn's test, $p = 1.73e-15$), and ML vs. TF (Dunn's test, $p = 1.27e-7$). In the Odra estuary, the total lengths of AG0 zander varied significantly between the stations (Kruskal-Wallis H-test, $p = 2.371e-5$). Zander from GB were significantly larger than specimens from OL (Dunn's test, $p = 0.0001$) and specimens from PB were also larger compared to OL (Dunn's test, $p = 0.002$). No significant differences were found between PB and GB (Dunn's test, $p = 0.154$).

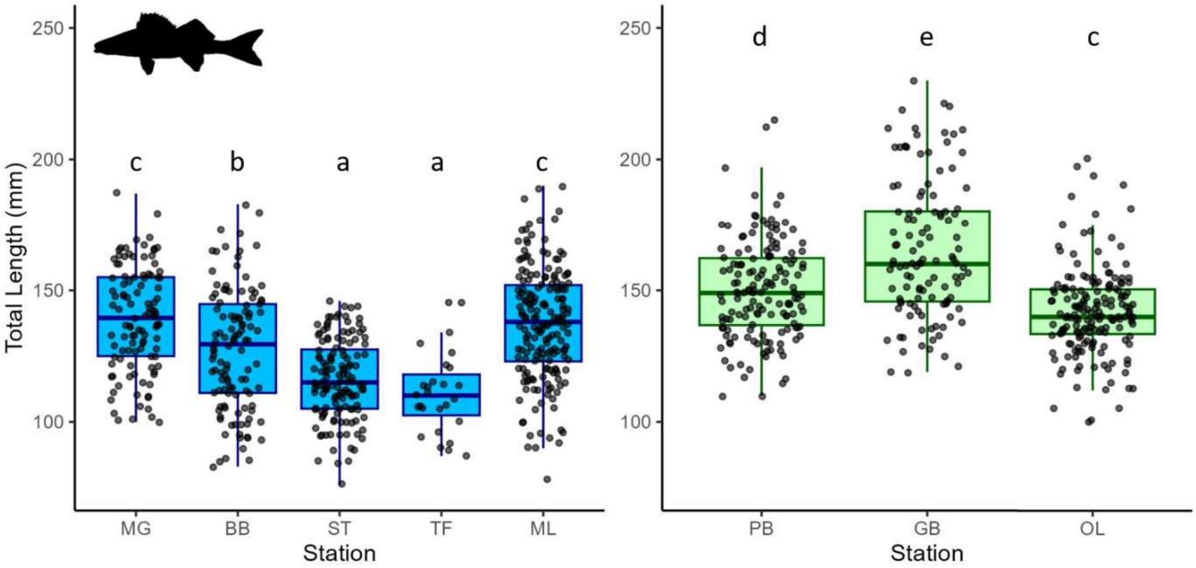

Figure 3.2: Total lengths of zander (*Sander lucioperca*) from the Elbe and Odra estuaries between 2021-2022. Elbe (left; n = 569), Odra (right; n = 439).

3.1.2 Smelt

In the Elbe estuary, the following median values and corresponding ranges were recorded for each age group (AG): AG0 of smelt: 76 mm (55–101 mm), AG1: 108 mm (75–154 mm), AG2: 154 mm (119–189 mm), AG3: 177 mm (134–226 mm), and AG4: 200 mm (172–221 mm). In the Odra estuary, the median values and ranges for each age group were as follows: AG0: 80 mm (72–87 mm), AG2: 164 mm (150–170 mm), AG3: 184 mm (169–219 mm), and AG4: 199 mm (196–202 mm). The calculated asymptotic total length for smelt from the Elbe estuary was 298.85 mm, while for specimens of the Odra the asymptotic length was smaller, at 238.65 mm (Fig 3.3). The growth coefficient was smaller in the Elbe (0.22) compared to smelt from the Odra (0.37).

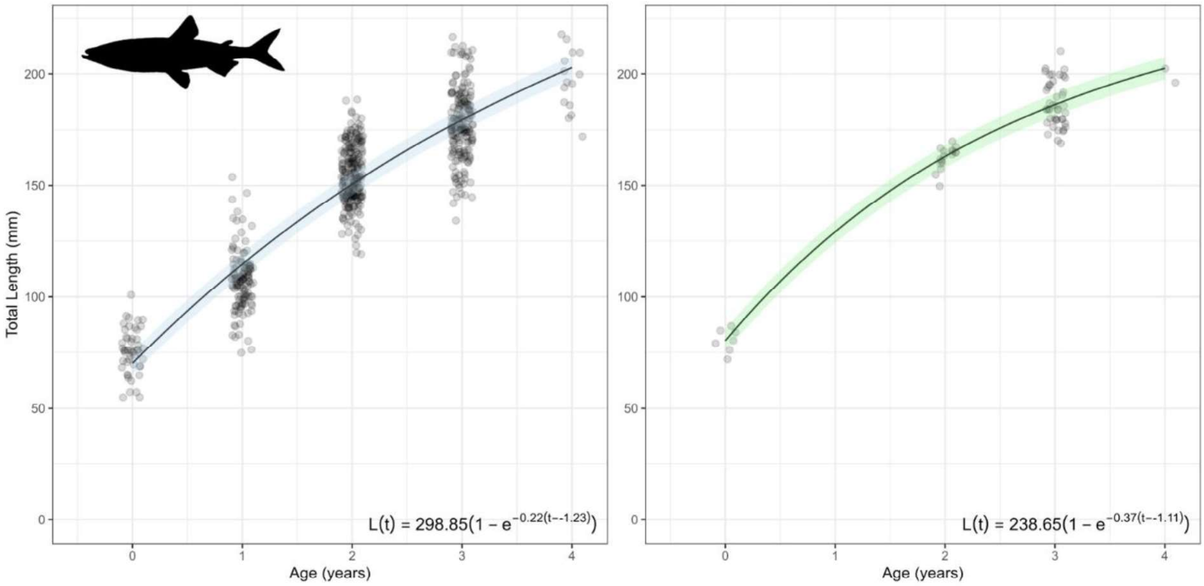

Figure 3.3: Von Bertalanffy growth curve for smelt (*Osmerus eperlanus*) from the Elbe and Odra estuaries in 2021-2022. Elbe (left; n = 645), Odra (right; n = 70).

3.1.3 Ruffe

A total of six age groups (AG0 to AG5) of ruffe were identified in the Elbe estuary. However, only a single individual with a total length of 190 mm was found belonging to AG5 and was therefore excluded from the comparative analysis between estuaries. In the Odra estuary, four age groups (AG0 to AG4) of ruffe were found. The following median values (and ranges) were determined for each age group in the Elbe estuary: AG0: 98 mm (50–129 mm), AG1: 126 mm (100–167 mm), AG2: 156 mm (119–177 mm), AG3: 181 mm (152–190 mm), AG4: 178 mm (149–220 mm). In the Odra estuary, the median values (and ranges) for each age group were: AG0: 98 mm (72–153 mm), AG1: 138 mm (113–167 mm), AG2: 155 mm (120–189 mm), AG3: 181 mm (152–190 mm), AG4: 194 mm (176–198 mm). Ruffe of AG1 had a significantly smaller total length in the Elbe estuary compared to the Odra estuary (Mann-Whitney-U test, $p = 0.0011$), whereas AG3 individuals in the Elbe estuary exhibited a significantly greater total length than those in the Odra estuary (Mann-Whitney-U test, $p = 0.0015$). No statistically significant differences in total length were observed between estuaries for AG0, AG2, and AG4 (Mann-Whitney-U test, $p > 0.05$). The asymptotic length of ruffe from the Elbe estuary was larger (219.60 mm) compared to 187.57 mm in the Odra estuary (Fig. 3.4). The growth coefficient was smaller in the Elbe (0.31) compared to specimens from the Odra (0.53).

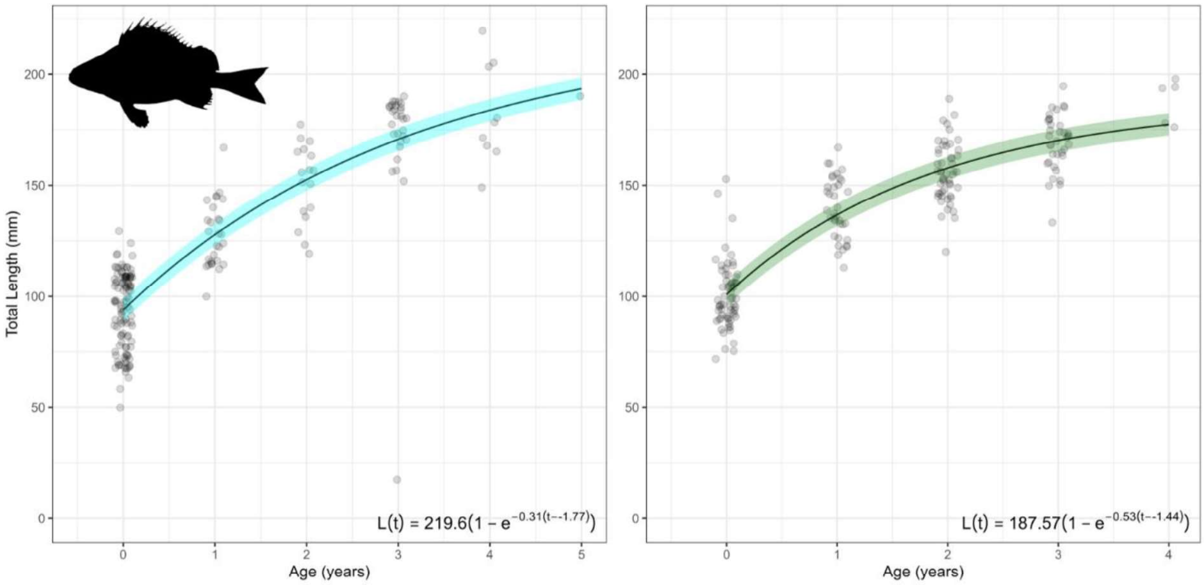

Figure 3.4: Von Bertalanffy growth curve for ruffe (*Gymnocephalus cernua*) from the Elbe and Odra estuaries in 2021-2022. Elbe (left; n = 192), Odra (right; n = 194).

3.1.4 Flounder

Both in the Elbe and Odra estuaries, five age groups of flounder (AG0 to AG4) were identified. In the Elbe estuary, the following median values (and ranges) were determined for the individual age groups: AG0: 72.5 (24–166) mm, AG1: 127 (65–339) mm, AG2: 264.5 (136–363) mm, AG3: 319.5 (216–383) mm, AG4: 313 (181–355) mm. In the Odra estuary, the median values (and ranges) for the individual age groups were: AG0: 102.5 (84–142) mm, AG1: 170 (92–297) mm, AG2: 242 (163–318) mm, AG3: 302 (129–375) mm, AG4: 310 (269–339) mm. Flounder of AG0 and AG1 had a significantly smaller total length in the Elbe estuary than in the Odra estuary (Mann-Whitney-U test, $p < 0.0001$ and $p = 0.0004$, respectively). No statistically significant differences in total length between the two estuaries were found for AG2, AG3, and AG4 (Mann-Whitney-U test, $p > 0.05$). The asymptotic total length calculated for flounder from the Elbe estuary was 854.71 mm, while in the Odra the asymptotic length was smaller (368.21 mm) (Fig. 3.5). The growth coefficient in the Odra was larger (0.34) compared to the Elbe (0.11).

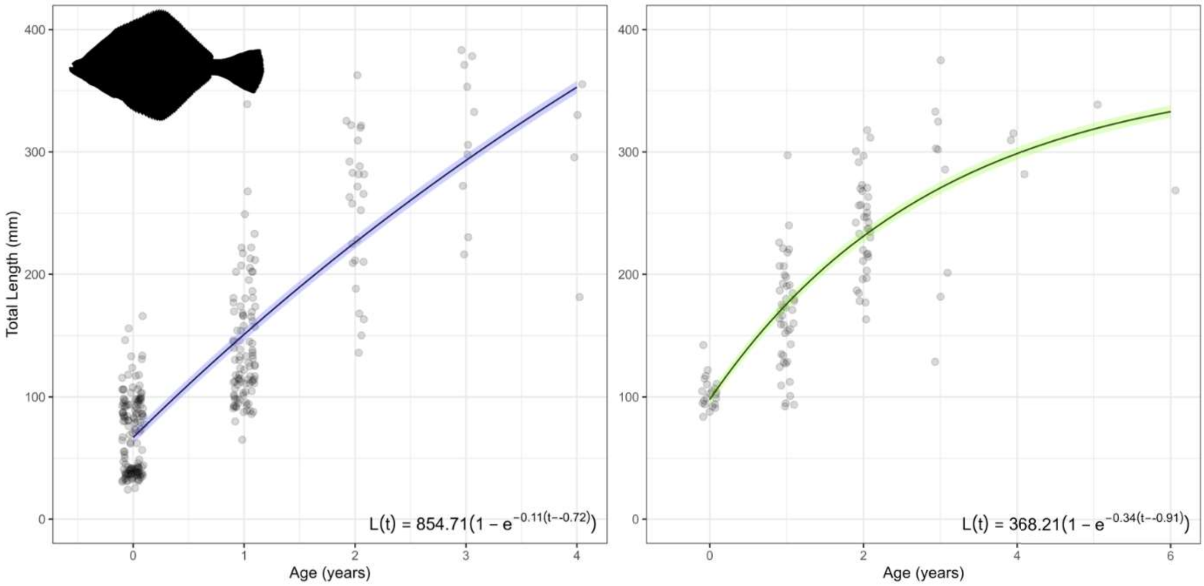

Figure 3.5: Von Bertalanffy growth curve for flounder (*Platichthys flesus*) from the Elbe and Odra estuaries in 2021-2022. Elbe (left; n = 284), Odra (right; n = 206).

3.2 Condition factor

Zander: In the Elbe estuary (n = 569), age-0 zander had the highest condition factors at the stations ML, MG, and BB, while the lowest conditions were recorded at TF and ST. Kruskal-Wallis H-test confirmed significant differences in condition factors between the stations ($p < 2.2e-16$). Specifically, the condition factors at BB were significantly higher than at ST (Dunn's test, p = 6.75e-13) and TF (Dunn's test, p = 0.003). Zander at MG had significantly higher condition compared to ST (Dunn's test, p = 1.47e-17) and TF (Dunn's test, p = 1.28e-4) as well. ML also had significantly higher condition factors than ST (Dunn's test, p = 1.59e-23) and TF (Dunn's test, p = 3.14e-5). In contrast, no significant differences were observed between BB vs. MG (Dunn's test, p = 0.491), BB vs. ML (Dunn's test, p = 0.256), MG vs. ML (Dunn's test, p = 0.774), or ST vs. TF (Dunn's test, p = 0.668). In the Odra estuary (n = 439), age-0 zander had the highest condition factors in the Greifswald Bodden, while lower conditions were recorded in the Pomeranian Bay and in the Odra Lagoon. Kruskal-Wallis H-test confirmed that significant differences in condition factors between regions were found (p = 8.71e-5). Specifically, condition factors at GB were significantly higher than at PB (Dunn's test, p = 0.0007) and OL (Dunn's test, p = 6.54e-5). However, no significant difference was observed between PB and the OL (Dunn's test, p = 0.531).

Smelt: The lowest condition factor was found at TF and the largest at BB (Elbe n = 645, Odra n = 70). This was confirmed by Kruskal-Wallis H-test (p = 0.01058), which showed significant differences between specimens from these two stations Dunn test BB vs. TF (Dunn's test, p = 0.00776).

Ruffe: The highest condition factors were found in the Odra estuary at GB and PB. Kruskal-Wallis H-test ($p = 2.12e-15$) showed that condition factors between stations differed significantly (Elbe n = 192, Odra n = 194). All combinations of stations between estuaries showed significantly larger condition factors in samples from the Odra estuary (except ML vs. OL, n.s.): BB vs. GB (Dunn's test, p = 0.0000000236), GB vs. ML (Dunn's test, p = 0.0002848), BB vs. OL (Dunn's test, p = 0.0102), BB vs. PB (Dunn's test, p = 0.00000576), ML vs. PB (Dunn's test, p = 0.00473), GB vs. ST (Dunn's test, p = 0.0000000262), OL vs. ST (Dunn's test, p = 0.00985), PB vs. ST (Dunn's test, p = 0.000000162), GB vs. TF (Dunn's test, p = 0.0000573), and PB vs. TF (Dunn's test, p = 0.000320). Within the Odra estuary condition factor of ruffe from GB and PB were significantly larger than OL:GB vs. OL (Dunn's test, p = 0.0000462), OL vs. PB (Dunn's test, p = 0.00275). In the Elbe estuary the condition factor of specimens from ML were highest and significant differences were found between BB vs. ML (Dunn's test, p = 0.0303).

Flounder: The highest condition factor was found at PB and GB in the Odra estuary (Elbe n = 284, Odra n = 206). The condition of specimens from those two station were significantly larger compared to specimens from the Elbe estuary: BB vs. GB (Dunn's test, p = 7.29e-11), GB vs. MG (Dunn's test, p = 1.10e-48), GB vs. ML (Dunn's test, p = 4.67e-104), BB vs. PB (Dunn's test, p = 3.45e-43), MG vs. PB (Dunn's test, p = 2.58e-65), ML vs. PB (Dunn's test, p = 1.38e-156), GB vs. ST (Dunn's test, p = 7.07e-31), PB vs. ST (Dunn's test, p = 3.84e-42), GB vs. TF (Dunn's test, p = 4.30e-24), PB vs. TF (Dunn's test, p = 2.27e-31). In the Elbe estuary the condition of flounder was largest at ML and smallest at BB and TF: BB vs. ML (Dunn's test, p = 6.53e-04), ML vs. TF (Dunn's test, p = 3.48e-03), while in the Odra estuary specimens from PB had better condition factors: GB vs. PB (Dunn's test, p = 4.76e-04).

4. Discussion

4.1 Growth of zander

Kafemann and Thiel (1998) reported on the growth of zander from multiple sources. For the same age group 0, the back-calculated total lengths in the Elbe estuary during the 1990s, were larger (15.1 cm) compared to overall mean total length from this study (12.9 cm) (Kafemann and Thiel, 1998). In the Kiel Canal, which is adjacent to the Elbe estuary, juvenile zander of AG0 reached a total length of 14.1, according to Kafemann and Thiel (1998). In the Ijssel (Willemsen, 1977) juvenile zander grew to 15.0 cm and in Lake Balaton they grew largest to a size of 20.4 cm (Bíró, 1970). Winkler (1980) calculated a total length of 16.9 cm for juvenile zander in the Greifswald Bodden. In this study, the mean length in the Greifswald Bodden was only slightly lower (16.5 cm). In the Odra Lagoon the AG0 zander of this study had a mean total length of 14.2 cm, which is smaller compared to findings reported by Wiktor (1957): 16.9 cm, Schlumberger (1962) 16.1 cm and Neuhaus (1934) 15.0 cm (Lehtonen et al., 1996). In the Curonian Lagoon (12.9 cm, Marre, 1933; 13.5 cm, Virbickas et al., 1974), Vistula Lagoon (12.2 cm, Filuk, 1955) grew less compared to the other water bodies (Lehtonen et al. 1996). In the river Stör (12.0 cm, Mohr, 1916) juvenile zander also grew less. Ložys (2004) concluded that a brackish environment has a beneficial effect on the growth of zander, which was also observed in this study, with the best growth found in the Greifswald Bodden (middle estuary). In contrast, the growth of juvenile zander in the brackish middle estuary of the Elbe was particularly lower.

Kafemann and Thiel (1998) showed in studies conducted in the Kiel Canal (NOK) that zander exhibited reduced growth due to the insufficient availability of their preferred prey (fish), particularly smelt (van Densen, 1985; Buijse and Houthuijzen, 1992) and herring (Winkler, 1980). The juvenile zander examined by Theilen et al. (2025b) showed slightly lower fish consumption in the Elbe estuary (IRB = 48 %) compared to the Odra estuary (IRB = 55 %) in the summer of 2021. This coincided with lower length growth in the Elbe estuary compared to zander from the Odra estuary. Within the Elbe estuary, juvenile zander at ST and TF stations exhibited both reduced length growth and lower condition factors compared to other stations (Koll et al., 2024). This reduced growth can be linked to a lower fish consumption rate relative to other stations. Instead, juvenile zander at these locations consumed proportionally more invertebrates (Amphipoda, Mysida, Decapoda) than at other stations (Theilen et al., 2025b). The reduced fish consumption at ST and TF can be attributed to a lower fish availability, particularly smelt. In summer 2021, the smelt density at ST was 6,905 individuals per 1 million m^3 and at TF, 9,894 individuals per 1 million m^3. In contrast, the downstream and upstream stations had much higher smelt densities (MG: 30,360 Ind./1Mio m^3, BB: 20,284 Ind./1Mio m^3, ML: 32,307 Ind./1Mio m^3) (Theilen et al., 2025a). Koll et al. (2024) showed that the zander in this region expressed starvation stress on a molecular level (transcriptomes). Additionally, the high turbidity at ST and TF may have negatively impacted the hunting success of juvenile zander. According to Amann et al. (2012), the zone of maximum turbidity (MTZ) in the Elbe estuary lies between Elbe kilometre (Ekm) 650 and 705, which includes the stations TF, ST, and BB. The exact location of the MTZ is dynamic and varies in response to hydrological conditions such as river discharge and tidal forces (Abascal-Zorrilla et al., 2020). Overall the growth of AG0 zander from the Elbe estuary was considerable smaller compared to other water bodies and previous studies, while zander from the Odra estuary grew only slightly less compared to studies from the Odra lagoon and Greifswald Bodden.

4.2 Growth of smelt

In the Elbe estuary, the asymptotic length (L_∞) was higher at 29.8 cm compared to 23.8 cm in the Odra estuary. In contrast, the growth coefficient (K) was higher in the Odra estuary (0.37) than in the Elbe (0.22). Wichmann (2017) calculated an asymptotic length of 23.8 cm and a growth coefficient (K) of 0.29 for smelt from the Elbe estuary, based on samples collected between December 2015 and March 2016.

In the late 1950s, average total lengths of smelt in the Elbe estuary were calculated for each age group (Trzebiatowski and Gaj, 1978), based on the data of Lillelund (1961): AG0 (7.1 cm), AG1 (13.4 cm), AG2 (17.3 cm), AG3 (21.2 cm), and AG4 (23.8 cm). In comparison, our study yielded a slightly higher mean for AG0 (7.5 cm), but lower mean lengths for subsequent age groups: AG1 (10.7 cm), AG2 (15.4 cm), AG3 (17.8 cm) and AG4 (19.9 cm). In the Odra estuary, we calculated a higher mean length for AG0 (8.0 cm), AG2 (16.2 cm) and AG3 (18.6 cm). No mean was calculated for AG1 due to a lack of samples and AG4 had the same mean in both estuaries (19.9 cm). Trzebiatowski and Gaj (1978) compiled data on smelt growth from various authors across Europe and Russia. In their work, they applied a different age group classification, in which the age group AG0 used in the present study corresponds to AG1 in their definition. Smelt populations reported by Trzebiatowski and Gaj (1978) exhibit high variability in age and size structure, with some populations reaching up to 30.1 cm at 12 years of age in the river Lena, as documented by Pirojnikov (1950). In the Odra Lagoon Krzykawska (1970) reported following means for AG1 (7.2 cm), AG2 (8.0 cm), AG3 (11.9 cm), AG4 (12.5 cm) and AG5 (13.5 cm). According to Wiórek (1968), smelt from the Vistula Lagoon grew to following size at the respective age group: AG0 (5.8 cm), AG1 (8.6 cm), AG2 (11.5 cm), AG3 (14.2 cm), AG4 (16.7 cm), AG5 (18.9 cm) and AG6 (20.9 cm). In the Lake Onega the growth of smelt was slowest with following means per age group: AG0 (6.3 cm), AG1 (8.8 cm), AG2 (9.4 cm), AG3 (10.6 cm), AG4 (11.0 cm), AG5 (11.3 cm), AG6 (11.8 cm) and AG7 (12.3 cm) (Stefanovska, 1957; Trzebiatowski and Gaj, 1978). Other Lake populations as in Lake Ladoga expressed more growth: AG0 (8.0 cm), AG1 (9.5 cm), AG2 (10.7 cm), AG3 (15.8 cm), AG4 (18.3 cm)

(Trzebiatowski and Gaj, 1978). Overall growth of smelt in the Elbe and Odra estuaries was superior compared to those from lake populations and of those from the Odra lagoon. In recent years, growth of smelt in the Elbe estuary has been inferior compared to previous studies, indicating a temporal shift in the ecosystem.

4.3 Growth of ruffe

Hölker and Thiel (1998) described the asymptotic length (L_∞) for multiple water types inhabited by ruffe: tidal estuary (25.3 cm) were largest followed by lakes (19.3 cm), nontidal estuaries (18.7 cm) and rivers (17.9 cm). In ponds and rivers ruffe grow to a L_∞ of 14.6 and 14.5 cm (Hölker and Thiel, 1998). The asymptotic length of ruffe calculated in this study for the Odra estuary (18.7 cm) aligns with the findings of Hölker and Thiel (1998) for non-tidal estuaries. For ruffe from the Elbe estuary the asymptotic length was 21.9 cm and therefore smaller compared to the finding from Hölker and Thiel (1998). Furthermore, Hölker and Thiel (1998) found that the growth coefficient (K) was highest in ponds and reservoirs (0.42 and 0.37, respectively). In tidal estuaries, the growth coefficient was smaller (0.28). In non-tidal estuaries, the growth coefficient was slightly higher (0.32). This also aligns with the results from this study, where the growth coefficient was larger in the nontidal estuary (Odra, K = 0.53) compared to the tidal estuary (Elbe, K = 0.31).

A direct comparison of ruffe growth between the Elbe and Odra estuaries did not reveal a clear pattern of reduced growth in either estuary. In the Elbe estuary, AG1 exhibited lower length growth, whereas AG3 showed higher length growth compared to the Odra estuary. No significant differences in length growth were found for the other age groups between the two estuaries. However, a slight decrease in growth compared to historic studies on ruffe in the Elbe estuary was observed compared to the 1980s and 1990s. For example, Hammer and Hölker (1994) reported an average total length of 20.3 cm for females and 20.7 cm for males in AG4, whereas in this study a median total length of only 17.8 cm (for both sexes combined) was determined for AG4. The maximum total lengths of ruffe cited by Möller (1988) and Hölker and Thiel (1998) were 29 cm and 25 cm, for the Elbe estuary. In contrast, the maximum total length recorded in this study for the period 2021–2022 was only 22 cm. Overall, the growth of ruffe from the Elbe estuary was inferior compared to the literature, while the findings from the Odra estuary reflected the finding for the respective habitats.

4.4 Growth of flounder

In the Elbe estuary, flounder from this study reached an asymptotic length (L_∞) of 85.4 cm with a growth coefficient (K) of 0.11, whereas in the Odra estuary, flounder reached an L_∞ of 36.8 cm but with a considerably higher growth coefficient of 0.34. In comparison, flounder from the Gulf of Gdańsk reached an asymptotic length (L_∞) of 69.1 cm with a growth coefficient (K) of 0.0915 (Antoszek and Krzykawski, 2005), while those from the Pomeranian Bay had a lower L_∞ of 54.7 cm but a slightly higher K of 0.1274 (Antoszek and Krzykawski, 2003). Flounder from the Odra estuary had a smaller asymptotic length but higher growth coefficient compared to studies from the Pomeranian Bay and Gulf of Gdańsk. Drevs et al. (1999) identified a significant decrease in flounder length per age group from southwest to northeast in the Baltic Sea. However, they also demonstrated that regional length differences became smaller as the flounder aged. Similarly, no statistically significant differences in total length were found between flounder from the Elbe and Odra estuaries for the older age groups (AG2, AG3, and AG4) caught during the 2021–2022 period. The unusually high L_∞ calculated by the VBGF (854.71 mm) eventually resulted from inaccuracies in age estimation. In general, age determination for flounder was less precise, particularly in the Elbe estuary where multiple specimens above 200 mm TL were assigned to AG1 and several specimens around 150 mm were assigned to AG0. In this study flounder from the Odra estuary, expressed a smaller asymptotic lengths but larger growth coefficient.

5. Conclusion

Overall, inferior growth was observed for zander in the Elbe estuary compared to the Odra estuary. For smelt, ruffe, and flounder, the asymptotic maximum length was larger in the Elbe estuary compared to the Odra, while the growth coefficient was smaller in the Elbe. Zander, smelt, and ruffe all showed smaller growth compared to previous studies, with zander particularly affected in the maximum turbidity zone of the Elbe estuary. The asymptotic length ($L\infty$) of ruffe also declined, while the age group means of smelt were smaller than previously reported. Flounder from the Odra estuary exhibited a smaller asymptotic length but a higher growth coefficient compared to studies from the Pomeranian Bay and the Gulf of Gdańsk. These findings suggest that changing environmental factors and food availability in the Elbe estuary may have influenced growth patterns, highlighting the need for continued monitoring to understand the underlying causes.

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8. Author Contributions

J.T.: conceptualization, investigation, field work, data collection and processing, statistical analyses, writing of the manuscript, design of figures, project administration. S.S., S.A.-M. and J.D.: investigation, data collection and processing, review and editing. E.H.: field work, investigation, review and editing. R.K.: field work, investigation, review and editing. C.M. and A.F.: conceptualization, funding acquisition, review and editing. R.T.: funding acquisition, conceptualization, supervision, review and editing, project administration. All authors have read and agreed to the published version of the manuscript.

Chapter 4 Environmental factors shaping fish fauna structure in a temperate mesotidal estuary: Periodic insights from the Elbe estuary across four decades

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Abstract

Anthropogenic perturbations paired with increasing climatic changes, affect the biota composition and ecosystem services provided by highly productive estuarine transitional ecosystems worldwide. To determine driving forces affecting fish stocks in an estuarine habitat, we created a periodic time series over the last four decades (1984–2022) combining fish species compositions and densities with environmental conditions along the course of the temperate mesotidal Elbe estuary. We detected major changes in the species composition alongside with changes in life cycle guilds composition. With a relative increase of marine-estuarine opportunists and reduction of diadromous species, the fish fauna of the Elbe estuary has become more similar in guild structure compared to macro tidal estuaries in Europe. Improvements in water quality in the 1990s were accompanied by increased fish densities, specially smelt (*Osmerus eperlanus*), until 2010. Anthropogenic hydromorphological interventions, however, could have led to an increase in suspended particular matter until 2022, which combined with reduced river runoff and poor oxygen concentrations in summer months acted as poor environmental conditions for fishes in the estuary. Mean fish densities dropped by over 91 % compared to 2010 to an all-time low in the data. This reduction was primarily a result of a decline of the key species smelt in the system along with declines of twaite shad (*Alosa fallax*), flounder (*Platichthys flesus*), ruffe (*Gymnocephalus cernua*), common bream (*Abramis brama*) and other species. On the contrary, marine species herring (*Clupea harengus*) and whiting (*Merlangius merlangus*) densities increased. Overall, the time-series provides insight into the strong impact of human intervention that are however expected to lead to further stressors.

Keywords: Estuary; Fish assemblage; Guild composition; Fish stocks; Environmental influences

1. Introduction

Estuaries constitute only 5.8 % of the coastal area (Wetzel et al., 2014), but are among the most important environments of the coastal zone providing habitats for numerous plant and animal species (Barbier et al., 2011). While the faunal composition varies due to spatial and temporal dynamics, estuaries are often characterized by high productivity (e.g. Thiel et al., 1995; Ducrotoy et al., 2019). However, environmental conditions of many estuaries worldwide are heavily influenced by humans (Ahlhorn, 2009) through impacts from industrial sewage, dyke construction, deepening of navigation channels and dumping of sediments to name a few (e.g. Barbier et al., 2011; Drabble, 2012; Wetzel et al., 2014). Today, estuaries are among the most heavily anthropogenically modified aquatic habitats on earth (e.g. Haedrich, 1983; Whitfield, 1996; Pein et al., 2023). Exogenous pressure is further exerted by climate change in particular (van Beusekom et al., 2018). Differences in the trends of climate change and human activities result in highly variable long-term developments (Ducrotoy et al., 2019), with anthropogenic factors generally influencing species richness, biomass, genetic structure and food webs in estuaries (Gilarranz et al., 2016).

The Elbe estuary, as one of the largest in Europe, shared a common fate of the above-mentioned interventions with estuaries worldwide (Pein et al., 2023), which has been shaped by a long history of human alterations (Bergemann et al., 1996; Schroeder, 1997; Kerner, 2007; Amann et al., 2012; Pein et al., 2021). Since the mid nineteenth century the Elbe estuary has been under anthropogenic influences, which includes channelization for shipping, increasing inlet of industrial, agricultural and communal sewage as well as abstraction of water for power plant cooling (Thiel et al., 2003; Kerner, 2007; Illing, 2009; Eick and Thiel, 2014). Hydromorphological alterations of the main channel had strong influences on the river runoff dynamics, tidal range and current velocities. The tidal range at the port of Hamburg (100 km upstream of the river mouth) doubled from 1.9 m to 3.8 m over the last century (Hein et al., 2021). As a further consequence of channelization, the sub- and intertidal shallow water areas, especially in the freshwater regions, were reduced. At the same time, oxygen deficiency situations in the Hamburg port region increase during summer months (Riedel-Lorjé and Gaumert, 1982; Thiel, 2011; Eick and Thiel, 2014; Scholle and Schuchard, 2020). However, the Elbe estuary still harbours internationally important ecological populations and habitats (Thiel and Thiel, 2015), like a number of other temperate estuaries worldwide (Ducrotoy et al., 2019).

The Elbe estuary serves as an important nursery habitat for several fish species. Especially the southern marginal areas of the estuary are characterized by very high fish production with over 200 kg/ha per annum (Thiel, 2001). These marginal areas provide shelter from predation by larger fishes due to shallower waters and reduced flow velocities and provide abundant food resources such as copepods (Eurytemora affinis) and mysids (Neomysis integer) (Sepulveda, 1994). These southern marginal areas were found directly downstream of the Hamburg harbour at Mühlenberger Loch (Ekm 633–636), Hanhöfer Nebenelbe (Ekm 636–644) and Lühesander Süderelbe (Ekm 647–652). Larval retention and accumulation of smelt was described by Sepulveda (1994), by active vertical migration, migrating to the surface layers during flood tides and to the bottom during ebb tides. Active vertical migration, upstream transport and accumulation in the southern marginal areas of the Elbe estuary was also described for flounder larvae (Bos, 2000). Besides providing essential feeding, spawning grounds and migration routes for a number of fish species (Nellen and Thiel, 1994; Thiel et al. 1996, 2003) the Elbe estuary with around 80 fish species is the most species-rich estuary in Europe (Thiel, 2011).

There is a lack of quantitative long-term studies on the influence of environmental and anthropogenic factors on the fish fauna composition in a number of European estuaries (e.g. Thiel, 2011) and estuaries worldwide (Pasquaud et al., 2015). A consistent and continuous dataset covering the entire period from the 1980s to the present day is still lacking, since evaluation of fish species composition in large estuaries is cost-intensive. Even when these data exist, there can be difficulties obtaining the data, since the data at the authorities/data portals are not always up to date. Monitoring data of fish composition and densities available from state funded monitoring used varying fishing methods rendering catch data from before 2000 and recent data not useable for this study. With the implementation of the Water Framework Directive (WFD) in 2000, fish surveys are conducted twice a year, however, these data are insufficient to study trends in fish communities since fish populations exhibit significant seasonal variations. Therefore, only data from stow net catches at five consecutive station were used, to insure best comparability.

To fill the knowledge gaps shown above, the aim of our study was to analyse historical and present compositions of the ichthyofauna in the Elbe estuary using a dataset of comparable traditional fishing methods incorporating seasonal sampling since the 1980s (Möller, 1988; Thiel et al. 1995, 2003; Thiel and Potter, 2001; Thiel, 2011; Eick and Thiel, 2014). The aims of the study include (1) the assessment of changes in the life cycle guilds, (2) the quantification of changes in the densities of the fish species and (3) the identification of long-term shifts of environmental factors and their influence on the fish species densities. In this regard, the Elbe estuary can be seen as a case study for other temperate mesotidal European estuaries, as it is one of the few estuaries in Europe for which quantitative data for fish and relevant environmental factors are available over longer time series.

2. Material and methods

2.1. Study area and stations

The study was conducted in the Elbe estuary, which is located in north-west Germany and ranging from a weir at the city of Geesthacht downstream to its mouth into the southern North Sea, close to the city of Cuxhaven. The strongly tidally influenced Elbe estuary is classified as mesotidal, coastal-plain estuary and has no marked halocline (Thiel and Potter, 2001; Eick and Thiel, 2014; Pein et al., 2023). In past studies, the Elbe estuary was categorized as a well-mixed estuary (Muylaert and Sabbe, 1996; Amann et al., 2012; Eick and Thiel, 2014), but recent findings from Pein et al. (2021), argue that hydrodynamic modelling approaches showed buoyancy driven density gradients, that induce periodic stratification, especially during summer. In extreme cases water temperature reaches above 25 °C during summer months and down to −1 °C in extreme winters (FGG-Elbe, 2024). The freshwater region (salinity <0.5 PSU) extends from the Geesthacht weir (Ekm 586; upper end of tidal influence) to Stade (Ekm 654), the brackish oligohaline area (salinity 0.5–5 PSU) reaches down to Brokdorf (Ekm 684) and the brackish mesohaline area (salinity 5–18 PSU) continues downstream to the city of Cuxhaven (Ekm 725) (ARGE

Elbe, 2007). Turbidity in the Elbe estuary is high, and has increased in recent years, which is mainly driven by marine sediments brought upstream by increased tidal pumping and resuspension by extensive dredging for channel maintenance (Kerner, 2007; Schulz et al., 2023). Kappenberg and Grabemann (2001) described a variability of suspended particular matter (SPM) in the Elbe estuary, with a maximum turbidity zone (MTZ) located between Ekms 670 and 690 before the year 2000. Due to seasonal variation of river runoff, the SPM concentration varies throughout the year.

The hydromorphological changes by channel deepening were accompanied by increased tidal pumping and therefore increased sediment transport towards the Hamburg port (Pein et al., 2021). Scholle and Schuchard (2020) showed that the shallow water areas in the Mühlenberger Lock were reduced from 210.000 m^3 in the year 2000 to 113.000 m^3 in the year 2016 by construction and siltation processes (Scholle and Schuchard, 2020), which could have influenced the estuary's nursery function. The filling of a portion of the Mühlenberger Loch (2001–2004) led to a direct loss of shallow water areas in the southern marginal areas.

We selected five stations along the salinity gradient at regularly spaced distances within the Elbe estuary (Fig. 4.1): mesohaline (river mouth section): Medem Reede/Medemgrund at Elbe-kilometre (Ekm) 713/716 (A’/A), oligo-mesohaline (lower section): Brunsbüttel at Ekm 692 (B), oligohaline (middle section): Schwarztonnensand at Ekm 665 (C), freshwater-oligohaline (upper section): Twielenfleth at Ekm 651 (D) and freshwater (upper section): Neβsand/Mühlenberger Loch at Ekm 633/639 (E’/E). Out of these five stations, three stations (B, C, D) were sampled at the exact same location throughout entire investigation period, while stations A and E had to be slightly relocated (approx. 6 km difference) due to changed nautical circumstances and regulations.

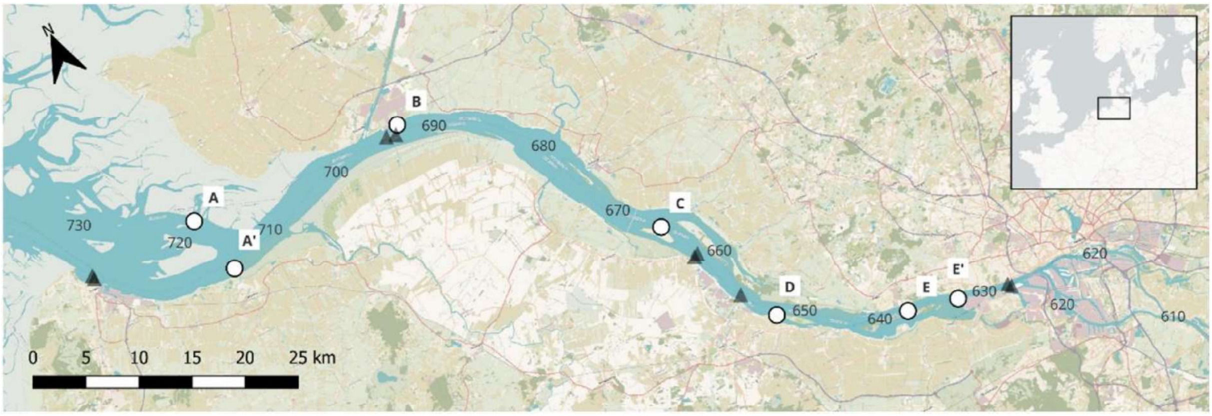

Fig. 4.1. Elbe estuary with the sampled stow net stations (white circles): Medem Reede/Medemgrund at Ekm 713/716 (A’/A), Brunsbüttel at Ekm 692 (B), Schwarztonnensand at Ekm 665 (C), Twielenfleth at Ekm 651 (D) and Neβsand/Mühlenberger Loch at Ekm 633/639 (E’/E). Stations from which environmental data were obtained from the FGG-Elbe data portal were indicated with triangles (Map sources: NextGIS, CartoDB).

2.2. Field sampling strategy

Sampling was conducted during four time periods (period I: 1984–1986, period II: 1994–1995, period III: 2009–2010, period IV: 2021–2022) covering all seasons (spring, summer, autumn, winter) in each period. Fishes were caught (day and night) using commercial stow net vessels having mesh sizes between 5 and 10 mm at the cod end (Fig. A1). The vessel was anchored at each sampling site and the net immersed, so that the net opening faced the direction of the water flow (Thiel et al. 1995, 2003; Thiel and Potter, 2001; Eick and Thiel, 2014). Species identification of the caught fish was conducted on board immediately. Fishes were counted, weighted (wet weight, 1 g accuracy) and total lengths were measured. Specimens that were not directly identifiable on board were either frozen using dry ice, freezers (−20 °C) or were preserved in formaldehyde solution (5 %) for later taxonomical identification in the laboratory following Whitehead et al. (1986), Muus and Nielsen (1999), Kottelat and Freyhof (2007), Knebelsberger and Thiel (2014) and Froese and Pauly (2022).

2.3. Harmonization of fish density data

In the initial time period I from 1984 to 1986, used stow nets had a net opening area of 80 m^2 and 95 m^2. One tidal phase (A at flood tide; B, C, D and E at ebb tide) was sampled per station (Möller, 1988), which resulted in one haul per sampling station at each sampling campaign. For comparison purposes, all catches in period I have already been converted to a net opening of 80 m^2 by Möller (1988). In time period II from 1994 to 1995 a stow net with a net opening of 90 m^2 was used (Thiel et al. 1995, 2003). During period II one stow net haul was performed during both tidal phases (flood an ebb tide) at each station resulting in two hauls per sampling station at each sampling campaign. In period III from 2009 to 2010 a stow net with a net opening of 135 m^2 was used (Eick and Thiel, 2014). During period III, during each tidal phase two hauls were conducted. For comparability these two hauls were summarized into one haul per each tidal phase. In period IV from 2021 to 2022 the stow net had a net opening of 135 m^2. The mean haul duration per period was 4 h for period I (Möller, 1988), 4 h during period II (Thiel et al., 2003), 3 h (combined) during period III (Eick and Thiel, 2014) and 3 h during period IV.

Fish densities were calculated based on the filtered water volume of each haul and the number of individuals of each species caught. The filtered water volume was determined by using the net opening area and water flow, which was measured using mechanical flow meters (Hydro-Bios® and/or General Oceanics®) during period II, II and IV. From the filtered water volume and the number of individuals caught was expressed as density, which was standardized to a fixed water volume (individuals per 1 million m3) for each haul by proportional rule. This standardization ensures consistency and comparability across hauls, regardless of the variations on the filtered water volumes. Water flow in period I was not measured directly but was approximated by using the haul durations of period I and comparable flow velocities from period II. In assumption that between period I and period II no major changes in the hydromorphology by channel deepening (Gaumert and Bergemann, 2007) of the Elbe estuary occurred, approximation of the water volume based on these two periods was regarded as sufficiently accurate. During period IV only the five sampling sites A-E were sampled and only one sampling campaign during each season was performed, whereas during the periods I (Möller, 1988), II (Thiel et al. 1995, 2003) and III (Eick and Thiel, 2014) a higher spatial and temporal resolution of the sampling data were available. In order to ensure the best possible comparability of the density data from the four different periods, the more detailed data records from periods I to III were reduced and adjusted to the resolution of period IV by utilizing records from the very same months.

2.4. Life cycle guilds and geographical distribution categories

Fish species in the Elbe estuary were grouped into life cycle guilds (LCG) following Thiel and Potter (2001), Thiel et al. (2003), Elliott et al. (2007), Franco et al. (2008), Thiel et al. (2011) and Eick and Thiel (2014): S: marine stragglers (e.g. lesser sand eel, *Ammodytes tobianus*), O: marine estuarine-opportunists (e.g. herring), E: solely estuarine (e.g. common goby, *Pomatoschistus microps*), C: catadromous (e.g. European eel, *Anguilla anguilla*), A: anadromous (e.g. smelt), F: freshwater (e.g. zander, *Sander lucioperca*). These definitions were based on the most recent work by Potter et al. (2013), who classified life cycle guilds of estuarine fish species worldwide. To ensure exact comparison with previous data (Thiel et al., 1995; Eick and Thiel, 2014), a classification with six LCG was chosen.

Freshwater fish species in the Elbe estuary were further grouped into two geographical distribution categories (Leroy et al., 2019): Palearctic fish species that have European, Siberian and East-Asian distributions and Nearctic fish species that are introduced from North America. Spatial and temporal variation in the fish fauna composition of the Elbe estuary can be described by their guild composition. A longitudinal change in the fish community in upstream direction corresponds with a decrease of densities of marine species and an increase of densities of freshwater species (Thiel, 2011; Eick and Thiel, 2014). In the past century, the anadromous smelt has been dominant fish species in the Elbe estuary since it occurs in the entire estuary with high densities and biomasses (Ehrenbaum, 1894; Lillelund, 1961; Möller, 1988; Thiel et al., 1995; Eick and Thiel, 2014). The invasive round goby (*Neogobius melanostomus*) was assigned to the freshwater LCG.

2.5. Collection of environmental data

Environmental data originated from long-term routine monitoring programs by the local authorities and were downloaded from the FIS Elbe data portal (FGG-Elbe, 2024). The variables electric conductivity (EC µS/cm: DIN EN 27888 C8), water temperature (wTemp °C: DIN 38404-4 C4), dissolved oxygen (DO mg/L and %: DIN ISO 17289 G25 and DIN 38408-23 G23), pH (DIN EN ISO 10523 C5), suspended particular matter (SPM mg/L: DIN EN 872 H33), ammonium (NH_4 mg/L: DIN EN ISO 11732:2005–05), nitrite (NO_2 mg/L: DIN EN ISO 13395 D28), nitrate (NO_3 mg/L: DIN EN ISO 10304-1 D20), phosphate (PO_4 mg/L: DIN EN ISO 15681-2 D46) and total organic carbon (TOC mg/L: DIN EN 1484 H3) were downloaded between the years 1982 and 2022. Monitored stations were chosen in closest proximity possible to the fishing sampling stations (see Appendix Tab. A.1).

The monitoring program of the local authorities included measurements from monthly helicopter flights (longitudinal profile) from 1982 to 1986, eight times a year from 1987 to 1993 (February, April, May, June, July, August, September, November) and six times per year from 1994 to 2022 (February, May, June, July, August, November). The water samples were extracted from the middle of the channel at a depth between 0.5 and 1 m during low tide (ARGE Elbe, 1999; FGG-Elbe, 2021). From the stations Brunsbüttelkoog (1982–2022), Grauerort (1982–2022) and Seemannshöft continuous measurements with usually one to four measurements per month were available. Monthly and annual means were calculated based on stationary continuous measurements and helicopter flights measurement.

2.6. Ordination and statistical analyses

Ordinations were computed using the R-package ‘vegan’ (Oksanen et al., 2022). Principal component analyses (PCA) was performed on standardized environmental variables (as percentage of maximum value recorded for the specific variable). Detrended correspondence analysis (DCA) was carried out on log10 transformed species density data was carried out for further data exploration (see Appendix Fig. A12). With the fist axis of the DCA expressing a gradient length >4, the data were suitable for unimodal analyses (ter Braak & Smilauer 2012). The relationship between fish species composition and environmental gradients were determined using unimodal canonical correspondence analysis (CCA). Statistical significance of the relationships between each environmental variable and the species composition were tested using the post-hoc permutation test anova.cca (999 permutations) of the ‘vegan’ R-package. Similarity percentage (SIMPER) analysis was carried out in order to identify species contribution to ordination. Analysis of similarities (ANOSIM) was used to determine differences of the fish fauna between periods, while using pairwise ANOSIM with Bonferroni correction to address differences between individual periods. Over the entire time series, fish density data from stow net hauls was assigned to environmental data by matching sampling month. Monthly median values of not normally distributed data were calculated for the ordination matrix of environmental factors. Hauls were removed from the dataset when no environmental data could be assigned. From originally n = 365 stow net hauls (period I n = 85, period II n = 72, period III n = 159, period IV n = 49) a total of n = 310 hauls was used for ordination computation (period I n = 79, period II n = 63, period III n = 126, period IV n = 42).

3. Results

3.1. Fish species composition

A total of 69 fish taxa were recorded across four time periods wherein period I (1984–1986) had 47 species, declining to 38 in period II (1994–1995), during period III (2009–2010) there was an increase to 53 species, that recently declined again to 44 species in period IV (2021–2022). During the entire investigation period, the 15 most abundant fish species were: smelt, herring, twaite shad, three-spined stickleback (*Gasterosteus aculeatus*), ruffe, flounder, sprat (*Sprattus, sprattus*), silver bream (*Blicca bjoerkna*), common bream, lesser pipefish (*Syngnathus rostellatus*), whiting, zander, common/sand goby (*Pomatoschistus* spp.), common seasnail (*Liparis liparis*) and European eel (Fig. 4.2, Tab. A.2). While smelt was the most abundant fish species in the Elbe estuary during all four time periods the ranks of the remaining species were different between the investigated time periods. Twaite shad was the second most abundant species during the 1980s, while in the 1990s twaite shad was on the 7th rank and during 2009–2010 it was on the 3rd rank and during recent survey (2021–2022) it was on the 11th rank. Herring displayed the third most abundant species during the 1980s and 1990s, the fourth most abundant species in 2009–2010 and the second most abundant species during the 2020s. Other abundant species were ruffe, which was the second most abundant species during 2009–2010 (1980s: 15th, 1990s: 5th, 2020s: 7th) and three-spined stickleback which was the second most abundant species during the 1990s (1980s: 4th, 2009–2010: 11th, 2020s: 9th). While whiting was the third most abundant species in the 2020s, the ranks of whiting were lower in the 1980s (42nd), 1990s (10th), 2009–2010 (26th).

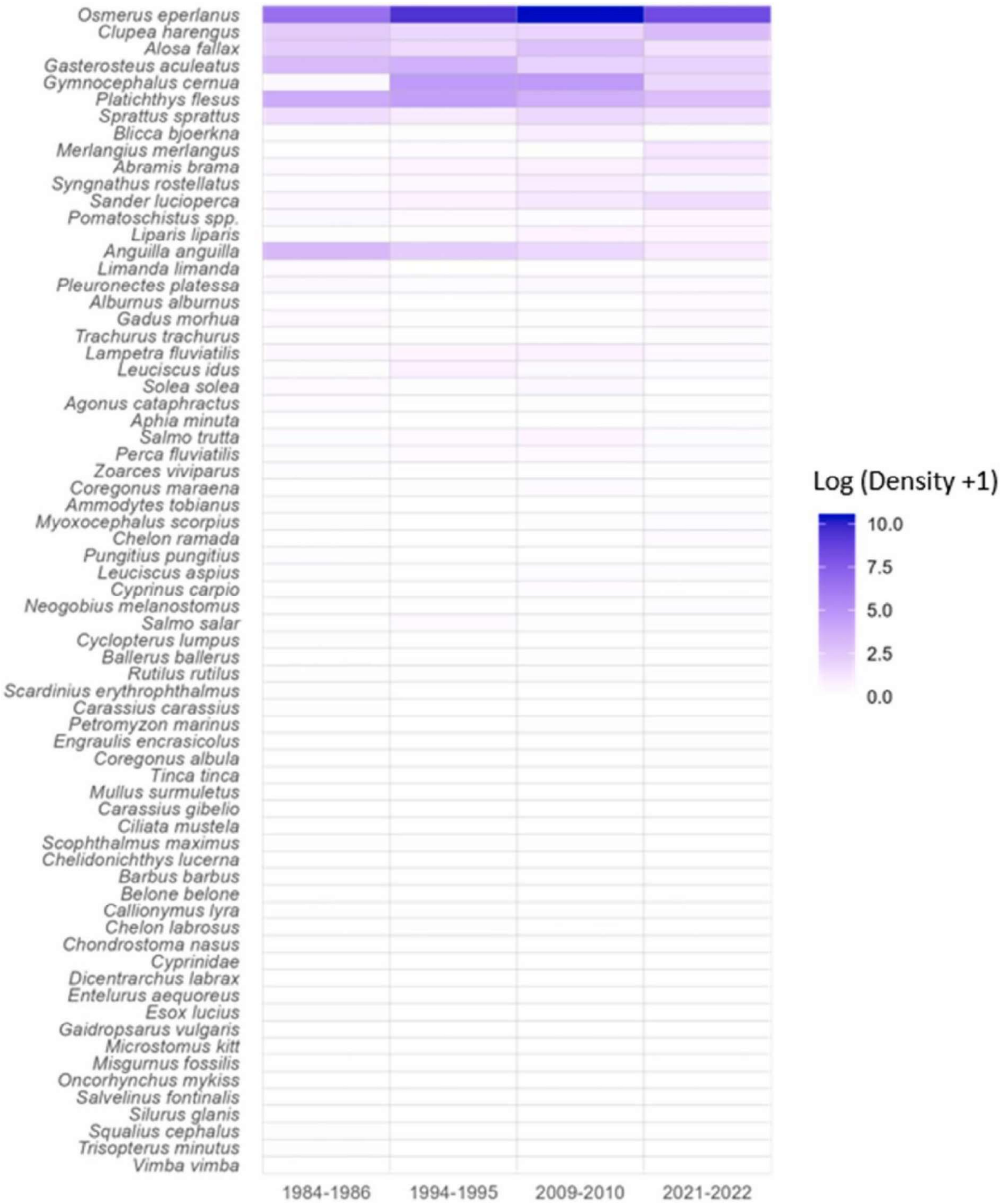

Fig. 4.2. Fish taxa found in in the Elbe estuary at the distinctive time periods 1984–1986, 1994–1995, 2009–2010 and 2021–2022. Changes of mean densities per haul (Log transformation) indicated by heatmap. Log-transformation of density data for enhanced visualization (large ranges of densities between species), untransformed data see Table A2.

The mean density per haul of several species exhibited varying patterns over time (Fig. 4.2, Tab A.2). Specifically, there was an increase in density observed from the 1980s through the 1990s, followed by a peak during the 2009–2010 period. However, our subsequent survey conducted in the 2020s indicated a decline in mean density of fish species such as smelt, zander, ruffe, river lamprey (*Lampetra fluviatilis*), silver bream, common bream and lesser pipefish in comparison with the previous period.

In the following we report the approximate number of fishes as individuals per 1 million m^3 water. Especially the mean density per haul of smelt reached from ca. 12,000 during the 1980s to 29,000 in the 1990s to 168,000 during 2009–2010 and was reduced with the most recent period (2020s) to 13,000. European eel expressed a gradual decrease over time, displaying the highest density during the 1980s and the lowest during 2020s. The density of twaite shad decreased from the 1980s from 1700 to 52 in the 1990s. With the period 2009–2010 density of twaite shad increased to 1000 and decreased with the latest period (2021–2022) to 12. Mean density of flounder increased in the 1990s up to 600, but exceeding to the time periods 2009–2010 (240) and 2021–2022 (33) the density was reduced. Common/sand goby had the highest density during the 1980s (165), which decreased to 15 in the 1990s and 0.7 during 2009–2010, while the density of common/sand goby increased in the time period 2021–2022 (26). Additionally, during 2021–2022 herring (1,500) and whiting (314) displayed their highest mean densities in the analysed time frame.

3.2. Spatiotemporal density variability of dominant fish species

Differences in the distribution of species density from the 1980s to the 2020s aim to illustrate variations in species dynamics, focusing on spatial disparities across five locations along the salinity gradient in the Elbe estuary. Boxplots for of fish species that were among the most abundant species (smelt, herring, twaite shad, three-spined stickleback, ruffe, flounder) as well as important predatory fish species (zander, whiting), river lamprey as endangered anadromous species and European eel as endangered catadromous species across various stations in the Elbe estuary were chosen to indicate their variance across spatiotemporal scales (Fig. 4.3).

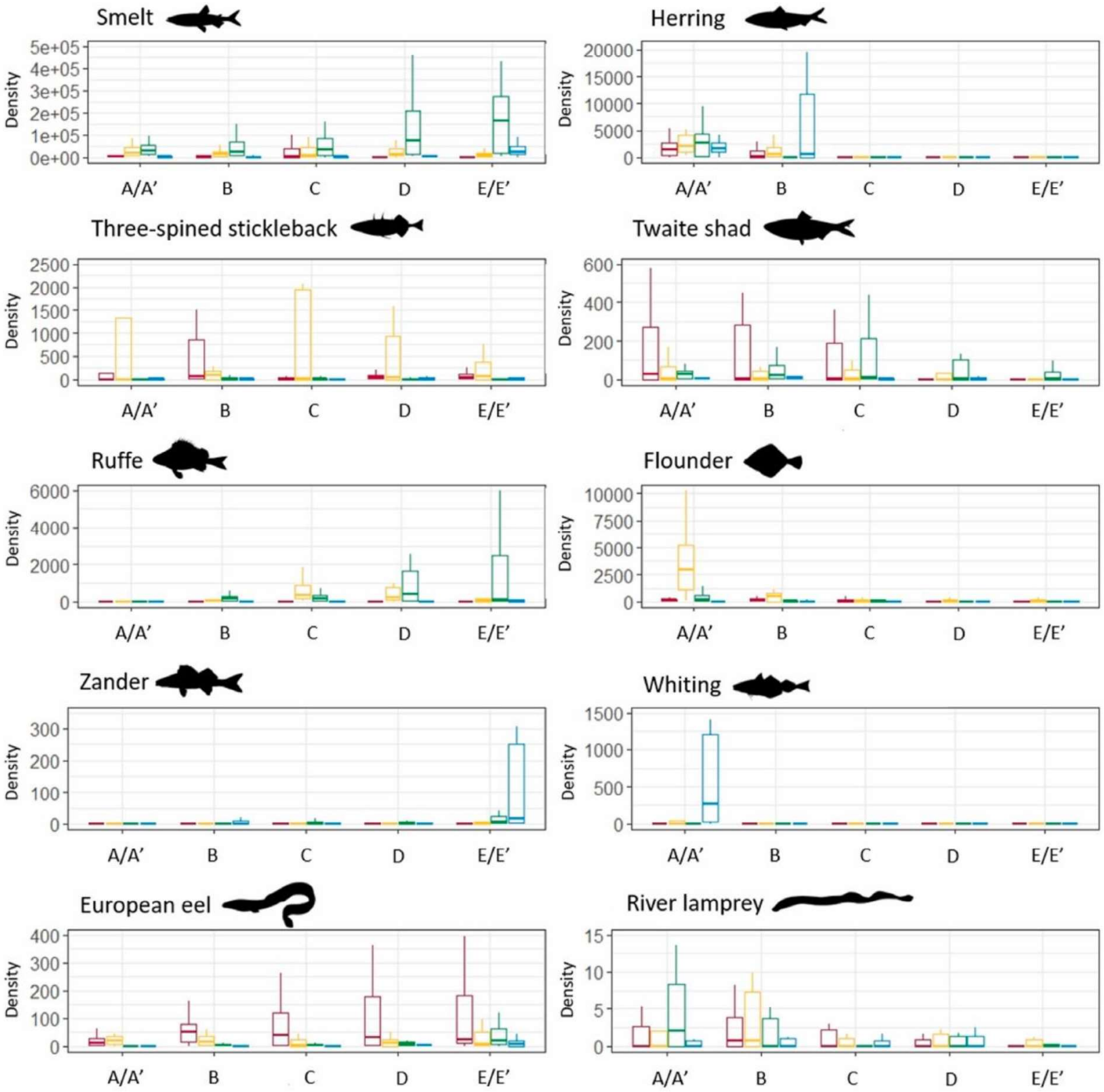

Fig. 4.3. Densities of ten of the key fish species along the longitudinal course of the Elbe estuary. A line at zero indicating complete absence of the species at the specific station during the referring time period. Medem Reede/Medemgrund at Ekm 713/716 (A/A'), Brunsbüttel at Ekm 692 (B), Schwarztonnensand at Ekm 665 (C), Twielenfleth at Ekm 651 (D) and Neβsand/Mühlenberger Loch at Ekm 633/639 (E/E'). Colours indicating the respective sampling periods: red (1984–1986), yellow (1994–1995), green (2009–2010) and blue (2021–2022). Outliers (>1.5 x interquartile range) removed for enhanced visualization of quartiles.

Densities of smelt, changed most substantially in the upstream regions being completely absent at several occasions during the 1980s ((Jun./Jul./Aug./Oct. 1985, Jun./Jul. 1986), D (May/Jun./Oct. 1985, Jun. 1986) and C (May 1986). In the most recent survey (2021–2022) the highest smelt densities were observed the Hamburg region. At E/E' with a median density of 25,000 during period IV, the population was reduced by nearly 85 %, compared to period III with a median density of 162,000. In the same time period the median density at D was reduced from 76,000 to 5000 while at C the density was reduced from 33,000 to 2200.

Generally, herring exhibited its highest median densities in the downstream areas, specifically at stations A/A' (1690 at period IV) and B (651 at period IV) near Cuxhaven and Brunsbüttel. Throughout all four time periods, herring was found up to the mid estuarine section (C). Density of herring was notably lower during the 1980s compared to surveys conducted in the 2020s, where the density reached its peak in that region. Only during the periods of 2009–2010 and 2021–2022 the presence of herring extended till the upper estuary (D), with higher density observed in the 2020s.

The highest median density (27) of twaite shad was recorded at A/A' during the 1980s, which had decreased to the 1990s (4.5), increased to 2009–2010 (26) and again decreased after the 2020 (4). During the 2020s, the highest maximum densities of twaite shad were observed at E/E' with 40 (spawners) and B with 262 (juveniles).

Three-spined stickleback density was highest during the 1990s period, especially at station C, where the median density of three-spined stickleback was17. During the most recent period (2021–2022) densities of three-spined stickleback were considerably lower at all five stations compared to period III (2009–2010).

During the 1980s, ruffe was absent in the downstream regions at A/A' and B, while in the 1990s, 2009–2010 and the 2020s ruffe occurred occasionally in low densities. However, during the 1990s and the 2009–2010 period, the density of ruffe was highest in the freshwater region, particularly at D and E/E'.

Flounder was found at its highest density during the 1990s with A/A' (median density: 3000). Lower median densities of flounder during the 1990s were found upstream at B (451), at C (43), at D (44) and at E/E' (23). Decrease of flounder over was most prominent in the downstream areas A/A' and B from period II to period III.

The highest densities of zander were particularly found close to the Hamburg area at E/E', during the 2009–2010 period. Zander were also most abundantly found at E/E' during the 2021–2022 survey. Between those two periods the median density of zander increased from 7.5 to 17 at E/E'. At the more downstream areas zander densities were lower during all four time periods.

Whiting was found exceptionally in the downstream regions in the river mouth at A/A' and B, where the higher densities were found at the outermost station A/A', especially during the 2021–2022 survey. During period IV at A/A' the median density of whiting reached 263, while the median density at the other periods was 0, since whiting occurred only sporadically during those time periods.

Generally, the density of river lamprey decreased with our recent sampling in the 2020s. The highest median densities of river lamprey were found in the river mouth are, especially during the 2009–2010 period at A/A' (2.1). At B the median densities were lower: 0.7 (1980s) and 0.8 (1990s). During period

III at D the maximum density of river lamprey recorded was 177, followed by 89 at C and 82 at D. The maximum density of river lamprey in the 2021–2022 period was found at B with 11, which had also decreased.

Densities of European eel decreased consistently at all five stations across the Elbe estuary over the four observed time periods.

3.3. Spatiotemporal variations of life cycle guilds

Marine stragglers were found only in the downstream areas (A/A' and B), with relatively low and consistent densities, with less than 0.1 % (Fig. 4.4, Tab. A.3). The highest density of marine stragglers, with a maximum of 1.6, was recorded during period IV (2020s) sampling at the downmost region (A/A').

Fig. 4.4. Spatiotemporal variation of mean densities of LCG at four distinct time periods along the salinity gradient of the Elbe estuary: Medem Reede/Medemgrund at Ekm 713/716 (A/A'), Brunsbüttel at Ekm 692 (B), Schwarztonnensand at Ekm 665 (C), Twielenfleth at Ekm 651 (D) and Neβsand/Mühlenberger Loch at Ekm 633/639 (E/E'). Log-transformation of density data for enhanced visualization (large ranges of densities between guilds), untransformed data see Tab A.3.

At A/A' the marine estuarine opportunists increased from 178 (1980s) to 597 (1990s) to 517 (2009–2010). From 2009 to 2010 to the 2020s at, the number of marine-estuarine opportunists decreased from 517 to 255. In contrast, the relative proportions between the 1980s and 2009–2010, remained nearly constant (8.6 %, 10.9 %, 7.6 %). However, compared to the most recent sampling relative proportion of marine estuarine opportunists increased from 7.6 % to 18.5 % at A/A'. At B, the number of marine estuarine opportunists from 1980s to the 1990s remained constant with densities of 220 and 191 (6.9 %, 5.1%), while from 2009 to 2010 to 2021–2022 marine estuarine opportunists increased from 116 to 464 at station B, which was a relative increase from 1.4 % to 36.9 %. In the middle section of the estuary the density of marine-estuarine opportunists devolved from 14.8 (1980s) over to 9.3 (1990s) to 12.9 (2009–2010) and 17.0 (2020s). Therefore, the relative proportion increased from 0.1 % to 0.3 % (1980s until 2009–2010) to 3.9 % during the 2020s. In the upper sections the densities of the estuarine-opportunists LCG were lower.

In the river mouth section (A/A') the density of the estuarine LCG decreased from 660 (1980s) to 99 (1990s), to 567 (2009–2010) and 12 (2020s), while in the lower section at B, densities decreased from

173 during the 1980s to 21 in the 1990s, 1.0 in 2009–2010 and 2.0 during the 2020s. Their numbers remained nearly constant at the freshwater to upper oligohaline areas with densities around 0.5 (<0.1 %).

The number of catadromous species consistently decreased throughout the entire estuary from the 1980s to the 2020s. The mean density of anadromous species increased from the 1980s to the 1990s and further to 2009–2010, but then drastically decreased in the 2020s. In the downstream areas, changes in anadromous LCG densities were less pronounced compared to the upstream areas. From 2009 to 2010 period to 2021–2022 period the density of freshwater species declined notable in the freshwater regions of E/E′ and D.

3.4. Long term shifts of environmental factors

Over the course of the past 40 years, the Elbe estuary underwent changes in environmental conditions (Fig. 4.5). EC in the upstream regions (D, E/E′) decreased from 1980s until the early 2000s, but increased from there slightly. The pH was lowest during the 1980s and early 1990s in the upstream regions (C- E). TOC had decreased in all five stations since the 1980s. At region C the total organic carbon concentration increased again. Phosphate concentration was highest in the upstream regions (D & E) during the 1980s and gradually decreased across the time scale. Nitrate concentration at all five regions decreased from 1980s until 2022. Maximum nitrate values were around 8 mg/L at region B during the early 1980s and early 1990s. Nitrite concentration was highest at region E/E′, especially during the entire 1980s decade, shown by highest annual means. At region E/E′ and B nitrite peaks above 0.8 mg/L. On multiple occasions nitrite concentrations above 0.5 mg/L were found at stations E/E′, D and C. Generally, the nitrite concentration decreased over time. The highest annual ammonium concentrations were found in the upper and middle sections E/E′, D, and C. The maximum concentrations in the upper and middle sections E/E′, D and C ranged between 4 mg/L and 5 mg/L during the 1980s. From the beginning of the 1990s the ammonium concentration decreased in the entire estuary.

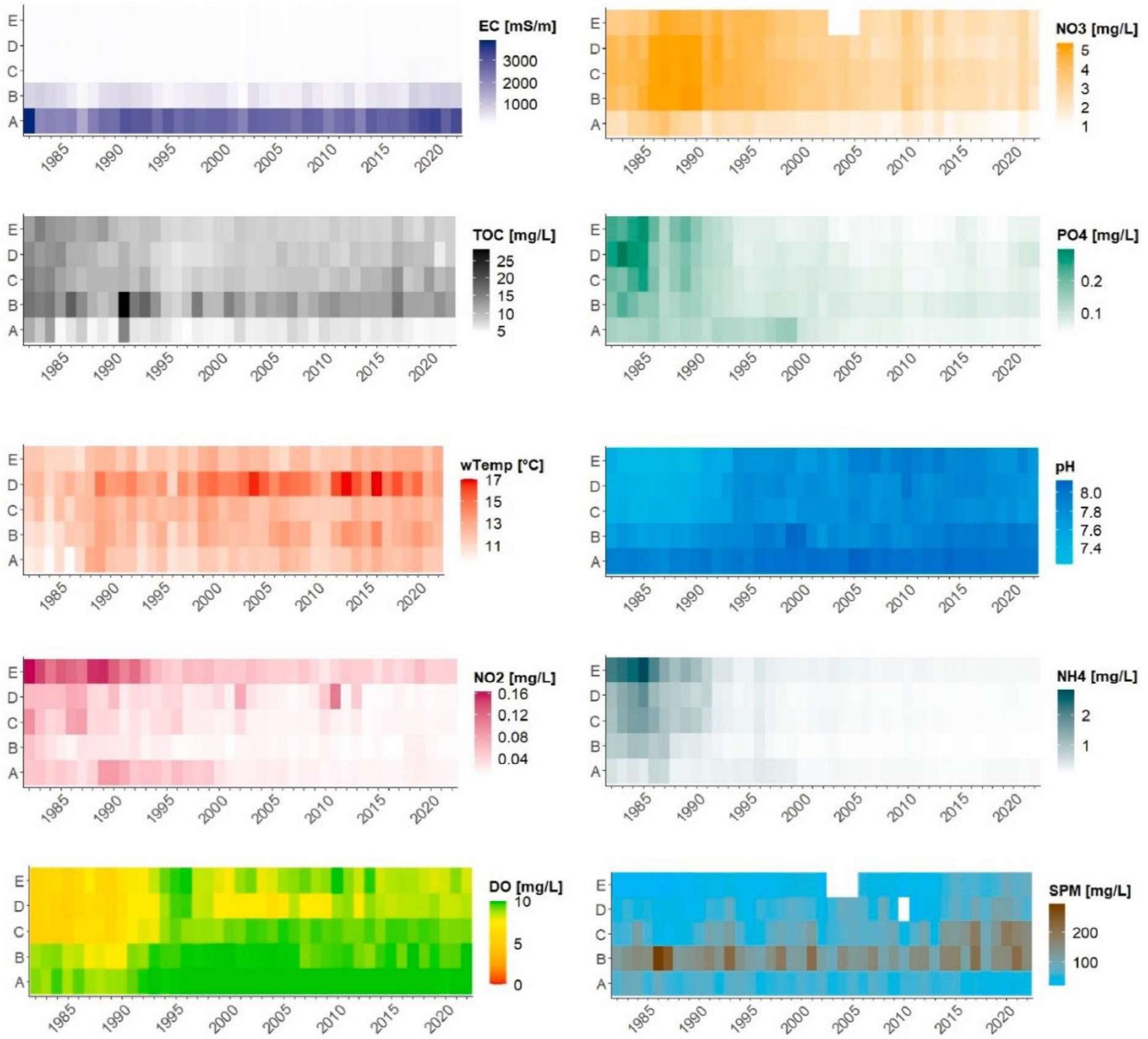

Fig. 4.5. Environmental parameter electric conductivity (EC), nitrate (NO_3), nitrite (NO_2), ammonium (NH_4), phosphate (PO_4), total organic carbon (TOC), suspended particular matter (SPM), water temperature (wTemp), dissolved oxygen (DO) and pH as heatmap (annual mean) from 1982 to 2022. A = Medem Reede/Medemgrund (Ekm 713), B = Brunsbüttel (Ekm 692), C = Schwarztonnensand (Ekm 665), D = Twielenfleth (Ekm 651), E = Neβsand/Mühlenberger Loch (Ekm 633).

The oxygen concentration in the Elbe estuary was particularly lowest during the 1980s in the upstream region E/E′, D and C, shown by the annual mean oxygen levels. In the river mouth at station A/A′, no critical oxygen values were measured at any time. At region B, especially during the 1980, critical oxygen levels were measured. In this region, on multiple occasions the oxygen concentration fell below 4 mg/L, with incidences below 2 mg/L in the early 1980s. Going further upstream lower oxygen concentrations were more frequently measured, which resulted in lower annual mean DO. At regions C and D oxygen concentrations below 2 mg/L occurred most frequently during the 1980s and oxygen situation improved from the 1990s on. At region E/E′, located in the Hamburg port area, oxygen concentrations close to zero were measured during the 1980s. Although the oxygen situation had improved from the 1990s onward, low oxygen levels were still regularly found in that region until the year 2022. During hypoxic events in that time period oxygen concentrations below 4 mg/L and 2 mg/L were measured, especially during summer months.

At the station at A/A′, located at the river mouth, the annual mean of SPM ranged from 38.1 mg/L to 89.7 mg/L. In the lower section at station B, the SPM measurements reached the maximum, with annual means ranging from 90.5 mg/L (2003) to 293.9 mg/L (1986). At station C in the time frame from 1982 to 2010 (a 28-year period), the annual mean of SPM exceeded 100 mg/L on four occasions: 1984, 1991, 1993, and 2001. However, in a shorter 12-year period from 2010 to 2022, 100 mg/L was surpassed nine times, peaking at 193.8 mg/L in 2020. In region D, the highest annual means of SPM were recorded in 2019 (111.3 mg/L), 2020 (107.7 mg/L), and 2017 (97.2 mg/L). In the region of E/E′ the annual mean of SPM concentration had more than tripled from 1982 to 2022. From 1982 to 2013 the SPM annual mean at station E/E′ exceeded a concentration of 50 mg/L only once in 2006 (50.3 mg/L), however since 2014, the annual mean concentration of SPM has consistently surpassed 50 mg/L each year. During the 1980s, concentrations ranged between 18.6 mg/L and 30.7 mg/L. Subsequently, in the 1990s, they increased to a range of 30.5 mg/L to 44.6 mg/L. In the 2000s, concentrations continued to rise, reaching concentrations between 31.8 mg/L and 50.6 mg/L. In the 2010s concentrations were varying from 26.3 mg/L to 72.5 mg/L, and in the 2020s, SPM peaked between 71.1 mg/L and 92.1 mg/L.

The PCA (Fig. 4.6) shows spatial and periodical separation on both axis by the environmental variables. The axis PC1 accounted for 30.4 % while PC2 accounted for 21.7 % of the variation. The samples show a spatial gradient with station A as the station in the river mouth mostly ordinated at −2.5, while freshwater station (C, D, E) were mostly ordinated in positive ordination space extending to above +7 on PC1. Samples from period I are clearly separated from the other periods due to changed environmental conditions.

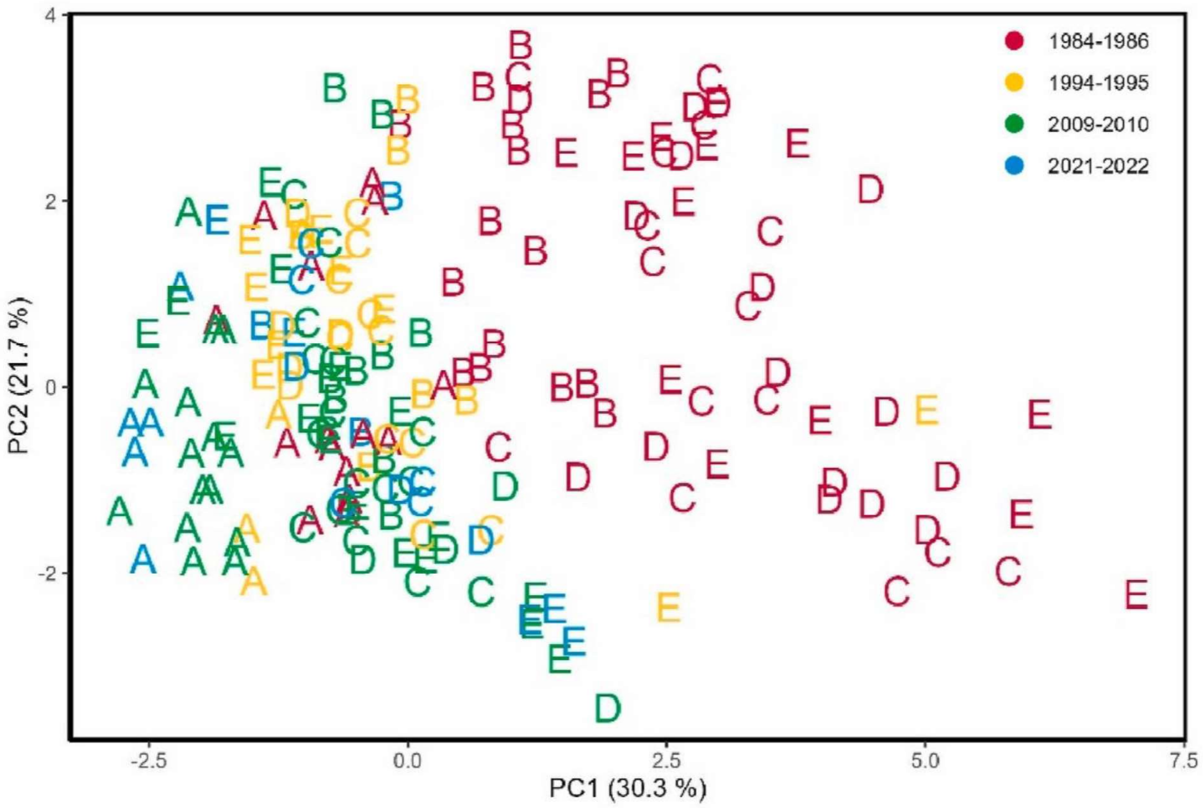

Fig. 4.6. Principle component analysis (PCA) classified by solely environomental variables measured in the Elbe estuary between 1984 and 2022. A = Medem Reede/Medemgrund (Ekm 713), B = Brunsbüttel (Ekm 692), C = Schwarztonnensand (Ekm 665), D = Twielenfleth (Ekm 651), E = Neβsand/Mühlenberger Loch (Ekm 633).

3.5. Influence of environmental factors on the fish fauna

The CCA ordination CCA1 (eigenvalue = 0.35) and CCA2 (eigenvalue = 0.12) accounted for 67.3 % of the variation (Fig. 4.7). The ordination showed the spatial distribution of the samples with stations in the river mouth (A, B) orientated in the positive ordination space along axis CCA1, while freshwater samples (D, E) were oriented in the negative ordination space. The fish species were oriented in a similar pattern: while marine stragglers e. g. hooknose (*Agonus cataphractus*) or tub gurnard (*Chelidonichthys lucerna*) and marine estuarine opportunists (e.g. herring, whiting) were oriented in the positive ordination space along CCA1 axis, freshwater species were orientated in the negative space. Diadromous fish species (e.g. smelt, twaite shad, three-spined stickleback) were ordinated centralized in the ordination space. This is also reflected by the salinity gradient as expressed by the vector EC in which the EC acts as a proxy for the salinity. Samples with higher water temperature are ordinated in the negative ordination space along CCA2 axis. The vectors for water quality parameters nitrate, nitrite, ammonium and TOC showed that samples from the 1980s mainly in the freshwater region (D, E) and the middle section (C) had higher concentrations compared to samples from the other periods.

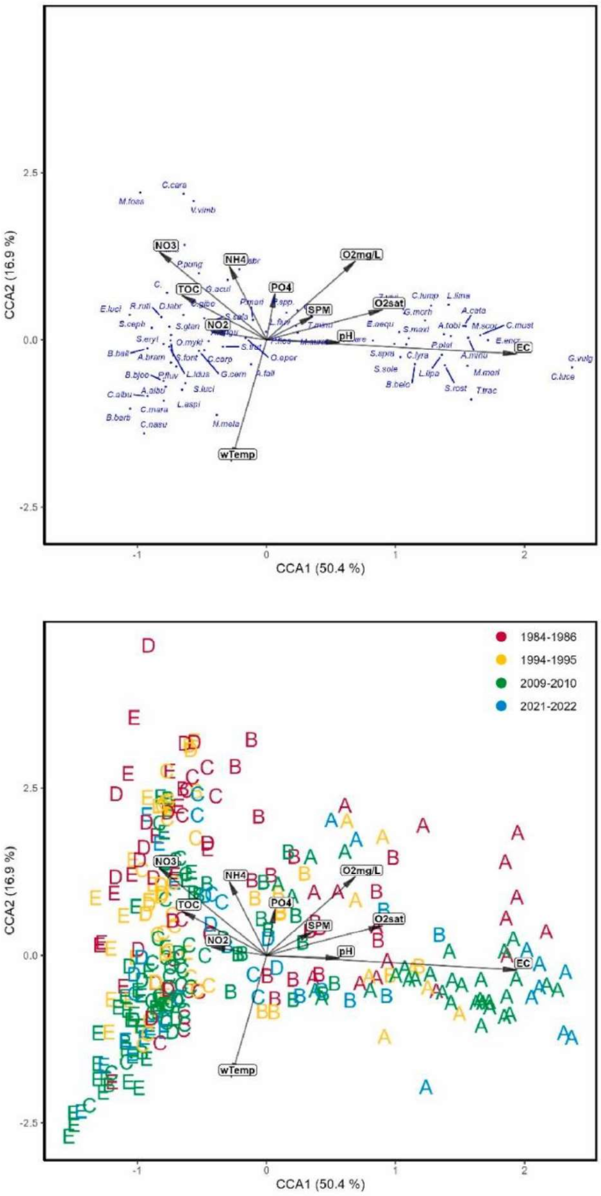

Fig. 4.7. Canonical correspondence analysis, separated for visualization by top: species as abbreviations; and bottom: environmental variables as vectors: EC (electric conductivity), SPM (suspended particular matter), NO_2 (nitrite), NO_3 (nitrate), NH_4 (ammonium), PO_4 (phosphate), TOC (total organic carbon), Wtemp (water temperature), O_2 (oxygen saturation in % and concentration in mg/L) and pH. ANOSIM ($R^2 = 0.219$, $p < 0.001$). A = Medem Reede/Medemgrund (Ekm 713), B = Brunsbüttel (Ekm 692), C = Schwarztonnensand (Ekm 665), D = Twielenfleth (Ekm 651), E = Neβsand/Mühlenberger Loch (Ekm 633). Species abbreviations: *Abramis brama (A.bram), Alburnus alburnus (A.albu), Alosa fallax (A.fall), Ammodytes tobianus (A.tobi), Anguilla anguilla (A.angu), Ballerus ballerus (B.ball), Carassius carassius (C.cara), Carassius gibelio (C.gibe), Chelon labrosus (C.labr), Chelon ramada (C.rama), Clupea harengus (C.hare), Coregonus albula (C.albu), Coregonus maraena (C.mara), Cyprinus carpio (C.carp), Dicentrarchus labrax (D.labr), Gadus morhua (G.morh), Gasterosteus aculeatus (G.acul), Gymnocephalus cernua (G.cern), Lampetra fluviatilis (L.fluv), Leuciscus aspius (L.aspi), Limanda limanda (L.lima), Merlangius merlangus (M.merl), Myoxocephalus scorpius (M.scor), Neogobius melanostomus (N.mela), Oncorhynchus mykiss (O.myk), Osmerus eperlanus (O.eper), Perca fluviatilis (P.flvi), Petromyzon marinus (P.mari), Platichthys flesus (P.fles), Pleuronectes platessa (P.plat), Pomatoschistus* spp. *(P.spp.), Pungitius pungitius (P.pung), Rutilus rutilus (R.ruti), Salmo salar (S.sala), Salmo trutta (S.trut), Salvelinus fontinalis (S.font), Sander lucioperca (S.luci), Scardinius erythrophthalmus (S.eryt), Scophthalmus maximus (S.maxi), Silurus glanis (S.glan), Solea solea (S.sole), Sprattus sprattus (S.sprat), Squalius cephalus (S.ceph), Tinca tinca (T.tinc), Trisopterus minutus (T.minu), Vimba vimba (V.vimb), Zoarces viviparus (Z.vivi).*

SIMPER analysis (Table 4.1) revealed that the largest influence on the ordination is caused by variation of density from smelt (21.5 %). The species that had the second most influence on the dissimilarity matrix was herring (8.9 %) followed by three-spined stickleback (7.9 %), ruffe (7.4 %), twaite shad (7.2 %), sprat (5.7 %), flounder (5.0 %), European eel (4.4 %), zander (3.5 %) and common bream (3.0 %).

Table 4.1: Cumulative percentages of the ten fish species responsible for the most variation (75 %) in the Elbe estuary (SIMPER-Analysis).

Species	**Cumulative contribution [%]**
Smelt	21.5
Herring	30.4
Three-spined stickleback	38.3
Ruffe	45.7
Twaite shad	52.9
Sprat	58.6
Flounder	63.6
European eel	68.0
Zander	71.5
Common bream	74.7

The four time periods 1984–1986, 1994–1995, 2009–2010 and 2021–2022 were statistically distinguishable by ANOSIM analysis ($R^2 = 0.219$, $p < 0.001$). Post-hoc pairwise ANOSIM with Bonferroni correction (adjusted alpha level reduced to 1/6th of 5 %) showed that dissimilarities between period I and IV were not significant ($R^2 = -0.01$, $p = 0.606$) as well as dissimilarities between period II and III ($R^2 = 0.05$, $p = 0.034$). Dissimilarities between period I and III were highest ($R^2 = 0.29$, $p = 0.001$), while dissimilarities between period III and IV ($R^2 = 0.17$, $p = 0.001$) or between period II and IV ($R^2 = 0.11$, $p = 0.002$) were also significant.

The post-hoc permutation test for constrained correspondence analysis showed that all factors had significant influence on the species composition (Table 4.2). Strongest influence was expressed by EC ($F = 41.91$), which acts as proxy for the salinity, since presence of marine species is highly depended on the salinity. Second strongest environmental variable was oxygen concentration ($F = 8.44$), since oxygen minimum zones had affected the species composition. Third and fourth strongest influences were expressed by nitrogen compounds nitrate (8.42) and ammonium ($F = 5.79$). SPM ($F = 5.33$) was identified as fifth most influential environmental variable affecting the fish species composition. Although being of minor importance, variables such as pH (F = 1.92) or TOC (F = 2.01) still had significant influence on the compositing of the fish fauna.

Tab. 4.2: Influences of environmental variables on the fish species composition by post-hoc permutation test. Higher F-value indicating more influence on the species composition.

Environmental variable	Chi-square	F-value	Pr(>F)
EC	0.33608	41.91	0.001***
O2mg/L	0.06767	8.44	0.001***
NO^3	0.06754	8.42	0.001***
NH^4	0.04646	5.79	0.001***
SPM	0.04275	5.33	0.001***
PO^4	0.03470	4.33	0.001***
NO^2	0.03325	4.15	0.001***
wTemp	0.02016	2.51	0.001***
O2sat%	0.02010	2.51	0.003**
TOC	0.01615	2.01	0.008**
pH	0.01541	1.92	0.021**

4. Discussion

4.1. Changes of life cycle guilds

In this study we aimed to describe changes in the density and the species composition in a temperate mesotidal estuary across four distinct periods extending over four decades and determine the driving environmental factors for fish occurrences.

As being among the most abundant species, smelt and twaite shad account for the largest portion of the anadromous LCG. Since the Elbe estuary provides suitable nursery areas for both species, we found juvenile life stages in high numbers. However, as both species have faced a reduction in their population sizes, the anadromous LCG decreased within recent years. From period III to period IV the mean density of the anadromous LCG was reduced from 20,000 to 1500 individuals per 1 million m3 resulting a decline of over 92 %. This LCG was mainly represented by smelt, twaite shad and three-spined stickleback.

The recent decline in the density of anadromous (juvenile) fish species in the Elbe estuary can be linked to a combination of factors contributing to the overall degradation of habitat quality. Key stressors include the recently increased oxygen deficiencies and SPM loads. Additionally, the reduction of shallow water areas within the freshwater region further increased the stress on growing larval and juvenile fish (Scholle and Schuchard, 2020; Illing et al., 2024). While tested environmental factors play significant roles, it is important to note that this decline is most likely a result of multiple interacting stressors. Further investigation with mesocosm experiments could further address these issues, in order to quantify the effects of multiple environmental stressors.

The catadromous LCG was mainly represented by European eel, whose populations decreased throughout Europe and therefore listed as 'critically endangered' on the IUCN Red List (Pike et al., 2020). The factors resulting in the collapse of European eel populations are versatile: migration barriers, silver eel mortality by hydropower plants, parasites e.g. swim bladder nematode *Anguillicola crassus*, disease spreading of anguillid herpesvirus by stocking, and fisheries (especially glass eel) (Behrmann-Godel and Eckmann, 2003; Briand et al., 2007; Dekker, 2016; Kullmann et al., 2017). These problems are not specifically related to factors found exclusively in the Elbe, but are found in other European water bodies as well.

The increase of marine species in the Elbe estuary can be explained by an increase of marine influence by the flood stream paired with reduced river runoff. The river runoff between 2014 and 2020 was 32 % lower than the long-term 1960–2020 mean (Rewrie et al., 2023). Due to enlarged channel diameter, larger quantities of marine waters can enter the Elbe estuary during high tides (Zhu et al., 2014; Ralston and Geyer, 2019), which favors the inland prevalence of marine fish species.

With the relative increase of marine-estuarine opportunists and the reduction of diadromous species, the Elbe estuary has become somewhat more similar in its guild structure to the macrotidal estuaries found in the UK (i.e. Forth, Tyne, Humber). In these estuaries the ecological guilds of estuarine residents, marine juvenile migrant and marine adventitious species displayed the largest portion of the fish fauna, while diadromous fish species were of less importance (Elliott and Dewailly, 1995). These alterations suggest an ongoing transition of the fish guild composition towards those found in a macrotidal estuary.

4.2. Environmental factors structuring the fish fauna

Over the entire time scale, certain environmental factors had a major influence on structuring the fish species composition in the Elbe estuary. The strongest influence was attributed to EC, which acts as a proxy for salinity. Stations with higher salinities at the river mouth and lower sections (A and B, see Fig. 4.1) had higher densities of marine stragglers and marine-estuarine opportunists, which confirms the findings of the spatiotemporal structure found in previous studies (e.g. Thiel and Potter, 2001; Eick and Thiel, 2014). With increasing sea level rise and reduced river runoff due to climate change, these marine influences are likely to increase even more in the future (Little et al., 2022; Pein et al., 2023).

Seasonal changes of the fish fauna composition in relation to water temperature were also described by Thiel and Potter (2001). With diadromous species such as smelt, twaite shad, three-spined stickleback in the fish community, species composition changes during migration periods and juvenile nursery time. Smelt densities peaked between early June and late August, when the population consists predominantly of juvenile young-of-the-year specimens. Densities of three spined-stickleback tended to be highest in colder temperatures during winter and early spring samples. In the freshwater sections common bream densities tended to be higher at warmer temperatures during summer months. Upstream migration of anadromous fish can be induced by factors such as rising water temperature (Aprahamian et al., 2003) and increased river runoff (Lillelund, 1961). The seasonal variation of the fish species composition is characterized by spawning and nursing activities, with high densities of juveniles occurring in the estuary during spring and summer. As water temperature follows seasonal patterns, the significant influence on the species composition expressed in Fig. 4.6, reflects these seasonal changes rather than long-term shifts by increased temperature.

In the 1980s water quality in the Elbe estuary was poor due to waste water sewage (ARGE Elbe, 1984; Möller and Scholz, 1991), which was expressed by high concentrations of nitrogen compounds (ammonium, nitrite, nitrate), TOC and phosphates. The concentrations of those compounds were highest in the freshwater and upper oligohaline areas of the estuary (Fig. 4.2). Nitrite is particularly toxic to fish by reducing oxygen uptake ultimately causing death (Russo and Thurston, 1977) by suffocation which is even more unfavourable during events with low oxygen concentration. Schäperclaus (1979) described concentrations of nitrite of 1 mg/L as non-toxic (with regard to lethal exposure). However more recent studies investigating long-term effects of sublethal nitrite exposure of 0.3 mg/L for 60 days indicated significantly lower food intake and growth performance in juvenile percids (Zhang et al., 2021). With values regularly exceeding 0.3 mg/L in the freshwater regions during the 1980s these values have been exceeded for long times in the Elbe estuary classified as highly polluted (LAWA, 1998). Adding to this, ammonium values that, depending on temperature and pH becomes fish toxic ammonia, reached values of 2.4 mg/L during multiple years rendering the estuarine waters as severely polluted by classification of LAWA (1998).

The water quality described in the 1980s was accompanied by recurring low oxygen concentrations, occasionally even anoxic conditions (Möller and Scholz, 1991). During anoxic events in the years 1985 and 1986, *O. eperlanus* was completely absent from E/E' (seven samples), D (four samples) and C (one sample) between May and August. Entire smelt generations can be destroyed by an early onset of oxygen depletion in their life cycle (Möller and Scholz, 1991; Thiel et al., 1995). During 1985 highest catches of juvenile smelt were in areas with more than 5 mg/L DO (Möller and Scholz, 1991). In the 1980s the oxygen minimum reached downstream to B, where values below 2 mg/L were measured.

After the 1990s at C and D no DO concentrations below 2 mg/L were measured, but occasionally values below 4 mg/L were reached. Generally, the oxygen situation improved compared to the 1980s, especially at B, C and D. Although the oxygen situation had improved after 1990, at E/E′ concentrations below 4 mg/L and 2 mg/L were still regularly measured during summer, meaning the oxygen situation at E/E′ remained relatively unchanged after the late 1990s. With regard to fish larvae that use the region around the Mühlenberger Loch (E/E′) as feeding area, Sepulveda (1994) showed that DO concentrations below 4.5 mg/L already reduced the growth performance of smelt larvae.

4.3. Anthropogenic influences on fish densities

Historically, nutrient loads and eutrophication have severely impacted fish populations, leading to significant oxygen depletion and causing destruction of entire cohorts (Möller and Scholz, 1991). As water quality improved, fish stocks had recovered between the mid-1990s and early 2000s. However, compared to the time period 2009–2010, our most recent survey showed that several of the dominant fish species in the Elbe estuary declined in their density.

In a scenario of intensified river dredging, the effects of tidal pumping are amplified, resulting in several stress factors for the fish fauna in the Elbe estuary. Bilkovic (2011) found that recently dredged tidal creeks provide less suitability nursery habitats for fish, while Yang et al. (2019) concluded that suspended sediments can have direct impacts on the survival of fish eggs and larvae. Model calculations showed that channel deepening appeared to be the main enhancing factor for an upstream transport of sediments (van Maren et al., 2015). Sexton et al. (2024) concluded that ship traffic in freshwater waterways is related with declines in biodiversity for fish and macroinvertebrates. They concluded that channelization and riparian degradation have a detrimental effect on these ecosystems. Findings from Thiel and Pezenburg (2007) showed that elevated SPM concentration in the Mühlenberger Loch region can induce alterations in the gill epithelial tissue (increase of mucocytes) of juvenile smelt and therefore could cause raised mortality rates and stunted growth.

Hecht and van der Lingen (1992) found that turbidity affects feeding behaviour in fish. Under different turbidity conditions, changes in feeding strategies were described, whereby visual predators are more affected by turbidity than fish that feed on macroinvertebrates. Higher concentrations of SPM were shown to affect lower trophic levels, resulting in less phytoplankton production by light limitation (de Jonge et al., 2014; Steidle and Vennell, 2024) leading to lower densities of zooplankton and benthic invertebrates (Shoup and Wahl, 2009) and therefore restrict food availability for fish. This is supported by findings from Bernát et al. (1994), who found that increased SPM negatively affects the filtration rate of calanoid copepod *Eurytemora affinis* in nauplii stages and adults, thus affecting copepod densities in recent years and disrupting the entire food web of the estuary. The increased turbidity in this scenario can affect the fishes in the Elbe estuary on various levels: firstly, the suspended solids could have caused irritation in the gill tissue, secondly, foraging behaviour could have been impaired and finally, overall food availability could have been reduced.

Increasing SPM concentration at C (Schwarztonnensand) showed that the reach of the maximum turbidity zone has migrated upstream. In the description of Lang (1990), in a mesotidal estuary the highest concentrations of SPM are found in the gradient zone, while in a macrotidal estuary higher concentrations of SPM can be found in the freshwater areas. With the increased tidal amplitude and the upstream migration of the MTZ, the Elbe estuary is driven towards a macrotidal estuary. Upstream migrations of the MTZ have already been observed in other heavily modified estuaries such as the Ems estuary (de Jonge et al., 2014). Especially the side channels of southern marginal area in the upper section of the estuary (between D and E/E′) Hahnöfer Nebenelbe, Lühesander Süderelbe and the shallow bay Mühlenberger Loch were described as locations where the annual production of fish was the highest (more than 200 kg/ha per year) in the entire estuary (Thiel, 2001). In the shallow water, the ratio between surface and water volume is in favour for the fish larvae, since diffusion of atmospheric oxygen and penetration through the shallow water column mitigates low oxygen situations, which occur in the main channel during summer. Copepod densities are highest in the Mühlenberger Loch area around E/E′ (Fiedler, 1991; Kafemann et al., 1996; Thiel, 2001), making this area the favoured nursery habitat for fish larvae, as prey organisms of the optimal size are available here. The upstream sediment transport from tidal pumping also carries organic compounds. These particles include organic carbon and active microorganisms that consume and break down the organic compounds (Tobias-Hunefeldt et al., 2024), leading to higher oxygen demands and oxygen deficiencies during summer. These oxygen deficiencies, especially in the freshwater regions of the estuary, which are the nurseries for several fish species lead to a reduction of survival of fish larvae and juveniles.

Another factor that can affect the autochthonous fish fauna are invasive species. In 2008 the first individual of round goby (*Neogobius melanostomus*) was caught by commercial fisherman in the Elbe estuary (Hempel and Thiel, 2013). The high numbers of records and juveniles by the year 2012 and 2013 suggested the establishment of the species in the Elbe estuary. Round goby can negatively impact local fish populations by competition for habitats and food resources or direct predation of eggs and juveniles. But they have also incorporated in the food web by being part of the diet composition of predatory fish such as zander (Hempel et al., 2016). While adult predatory fish benefit from round goby as an easily available prey throughout the year, they may suffer from them at earlier life stages, since percid fish eggs were found in the diet of round goby in the Elbe at Hamburg and the adjacent Kiel Kanal (Giesler, 2015; Hempel, 2017).

While several fish species in the Elbe estuary suffered from negative impacts, the marine-estuarine opportunist LCG (e.g. herring and whiting) did benefit from changing environmental factors. The upstream shift of the upper brackish water boarder allowed them to access a larger area of the estuary more easily. Whiting is a lusitanian fish species and according to Thiel et al. (2025) densities of whiting have recently increased in the German North Sea areas. Although high densities of juvenile whiting are not an event that occur every year, with the increasing temperature in the North Sea and the Elbe estuary due to climate change, the appearance of large juvenile whiting schools in the Elbe river mouth is likely to occur more regularly.

5. Conclusion

Our long-term case study in the Elbe estuary demonstrates the significant influence of environmental factors, many altered by human impacts, on fish fauna composition in European estuaries. Despite improvements in water quality and oxygen conditions boosting fish densities in the 1990s and early 2000s, recent years have seen a dramatic decline. Increased SPM concentrations, upstream shifts in turbidity, reduced river runoff, and hypoxic summer conditions have severely affected fish stocks, with mean fish densities plummeting by over 91 % from 2009 to 2010 to 2021–2022, primarily impacting smelt populations. These findings highlight the critical importance of protecting estuarine nurseries to maintain ecosystem services, which are currently undermined by ongoing ecological degradation. For key species like smelt, the lack of refuge areas due to blocked upstream migration exacerbates their plight which is likely to be intensified by climate change effects.

6. References

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8. Author contributions

JT: conceptualization, investigation, field work (methodology), project administration, data curation, formal analyses, writing – original draft; VS: data curation, formal analyses, writing – review & editing; EH: field work (methodology), investigation, writing – review & editing; RK: field work (methodology), investigation, review and editing; CM, AF: conceptualization, supervision, funding acquisition, writing – review & editing; RT: funding acquisition, conceptualization, supervision, formal analyses, writing – review & editing, project administration.

Chapter 5 Network-based integration of omics, physiological and environmental data in real-world Elbe estuarine Zander

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Abstract

Coastal and estuarine environments are under endogenic and exogenic pressures jeopardizing survival and diversity of inhabiting biota. Information of possible synergistic effects of multiple (a)biotic stressors and holobiont interaction are largely missing in estuaries like the Elbe but are of importance to estimate unforeseen effects on animals' physiology. Here, we seek to leverage host-transcriptional RNA-seq and gill mucus microbial 16S rRNA metabarcoding data coupled with physiological and abiotic measurements in a network analysis approach to decipher the impact of multiple stressors on the health of juvenile *Sander lucioperca* along one of the largest European estuaries. We find mesohaline areas characterized by gill tissue specific transcriptional responses matching osmosensing and tissue remodeling. Liver transcriptomes instead emphasized that zander from highly turbid areas were undergoing starvation which was supported by compromised body condition. Potential pathogenic bacteria, including *Shewanella*, *Acinetobacter*, *Aeromonas* and *Chryseobacterium*, dominated the gill microbiome along the freshwater transition and oxygen minimum zone. Their occurrence coincided with a strong adaptive and innate transcriptional immune response in host gill and enhanced energy demand in liver tissue supporting their potential pathogenicity. Taken together, we show physiological responses of a fish species and its microbiome to abiotic factors whose impact is expected to increase with consequences of climate change. We further present a method for the close-meshed detection of the main stressors and bacterial species with disease potential in a highly productive ecosystem.

Keywords: Transcriptomics and Metabarcoding; Network analysis; Hypoxia; Holobiont; Estuary

1. Introduction

Coastal and estuarine environments have undergone significant changes in the past century due to overexploitation, habitat fragmentation, pollution, and species invasion (Lotze et al., 2006). Estuaries, as transitional ecosystems, exhibit intense variations in physicochemical conditions such as hydrology, salinity, turbidity, temperature, oxygen levels and habitat availability (Elliott and Quintino, 2007). Global change is expected to increase pressures on fish communities in estuaries, with a particular focus on the increasing frequency and intensity of oxygen minimum zones (OMZ), which have critical impact on fish physiology and distribution (Breitburg et al., 2018; Cottingham et al., 2018; Diaz and Rosenberg, 2008; Lauchlan and Nagelkerken, 2020; Sampaio et al., 2021).

Adding to this, the tidal Elbe as one of Europe's largest estuaries faces substantial pressure from centuries of construction projects including diking and improvement of access to the Hamburg harbor. Deepening and maintenance dredging of navigation channels resulted in the relocation of ca. 23 million m3 sediment in 2021 (WSV.de, 2023) of which a proportion was resuspended into the water column (Kappenberg and Grabemann, 2001). Low dissolved oxygen levels, including instances of hypoxia (<2 mg/L) in upstream areas, have been reported since the 1980s (Schöl et al., 2015). During summer, the otherwise well-mixed estuary (Middelburg and Herman, 2007) experiences stratification, particularly in the port area and deepened navigation channel. Under these conditions it is common to observe oxygen depletion and ammonium accumulation in the hypolimnic waters (Pein et al., 2021). Limited efforts have been made to address these issues.

Fish respond to changes in their environment through various mechanisms, including behavioral, morphological, physiological, and molecular adaptations, with severe stress ultimately leading to mortality (Richards, 2009). Avoidance reactions in fish (Roberts et al., 2009; Thiel et al., 1995) can be hindered by factors such as oxygen minimum zones, which restrict access to deeper, oxygen-rich areas (Diaz and Rosenberg, 2008). The first timeseries studies on one anadromous fish species (*Osmerus eperlanus*) in the Elbe estuary have revealed massive declines in abundance over the last decade attributed to multiple stressors comprising habitat loss, cooling water extraction, dredging, increased turbidity and recurring hypoxia (Scholle and Schuchardt, 2020).

In this study we use juvenile zander *Sander lucioperca* (Linnaeus, 1758) (0+ cohort) sampled in late summer 2021 from the Elbe estuary as a model organism to explore gene expression patterns and holobiont interaction linked to biometric parameters indicative for physiological condition in response to steep abiotic gradients. The zander is among the most abundant fish in the ecosystem and among the top predatory species in the Elbe exerting a strong top-down control on other species (Eick and Thiel, 2014). In contrast to their embryonic stages, which require a salinity of <7 psu for their development (Klinkhardt and Winkler, 1989), juvenile and adult zander tolerate mesohaline conditions and strong osmotic fluctuations (Brown et al., 2001) allowing them to inhabit the entire estuary. Life history strategies of the population comprise either brackish residency or freshwater-brackish water strategy in which reproduction and larval growth occur in freshwater with later dispersal to more saline habitats (Kafemann et al., 2000). Larvae typically hatch between April and June and switch to piscivorous feeding within their first year (Vinni et al., 2009), with O. eperlanus being primary prey in the tidal Elbe (Kafemann and Thiel, 1998). The summer period is characterized by intensive growth, the juveniles sampled here spent 4 to 5 months in the estuary reaching 16 ± 2 cm with high metabolic activity susceptible to be altered by the multiple prevailing stressors (Laurent et al., 2023).

Despite a well-found knowledge of the abiotic and biotic changes occurring within the estuary, many spatio-temporal factors (i.e. temperature, salinity, DO levels) are confounding and make it difficult to determine the precise mechanisms underlying observed losses in fish biomass. Omics are increasingly considered in the context of biomonitoring and conservation biology, as they enable a deep understanding of processes at all levels of biological organization and of previously overlooked interactions (Connon et al., 2018; Miranda et al., 2023). Co-working of multiple stressors is expected to lead to unforeseen synergistic or antagonistic effects in organisms (Petitjean et al., 2019), sublethal impacts of which can contribute to population declines over generations (Connon et al., 2018). Molecular adjustments precede high level responses thereby probably allowing earlier detection of adverse situations (Bahamonde et al., 2016). Real-world omics studies indicate molecular mechanisms used by fish to cope with adverse effects of multi-stress environment. Reversely they can inform about the environmental conditions themselves allowing the identification of unexpected stressors as shown in recent studies (Jeffrey et al., 2019; Laurent et al., 2023).

Biotic interaction with microbiota on mucosal surfaces of teleost fish plays a crucial role in host metabolism, growth and health (Kelly and Salinas, 2017). The gill mucosal surface constitutes a special habitat for host-associated microbes due to its unique functions in waste excretion, gas exchange and immune activity (Pratte et al., 2018; Salinas, 2015). Local immune cell populations composed of adaptive and innate parts form the gill associated lymphoid tissue (GIALT) (Salinas, 2015) and enable fish to discriminate between beneficial and pathogenic bacteria thus keeping microbiome homeostasis (Yu et al., 2021). Changes in the environment however, including water conditions, seasonal changes and physiological stress can lead to compositional disturbances that turn commensals into pathogens (Kelly and Salinas, 2017). Several studies, particularly in aquaculture, have indicated an impaired immune response and spread of pathogens under low-oxygen conditions (Abdel-Tawwab et al., 2019; Boutin et al., 2013; Leeuwis et al., 2024). Likewise, suspended sediment was indicated to cause microbial shifts towards pathogenic communities (Hess et al., 2015). This sensitivity to environmental parameters makes the external mucus composition a biomarker for fish health built by a stable community of core taxa of low number but shared over a large range of conditions and a variable community strongly influenced the environment (Sehnal et al., 2021).

The objective of this study was to design an unsupervised network-based dimension reduction method based on real-world holobiont omics data from RNA and 16S rRNA libraries to precisely identify tissue-specific gene profiles and dynamics in bacterial composition. This allows us to determine driving stressors in the ecosystem and, by describing the microbiome and linking it to the immune response in the host, to provide a first basis for the use of microbial biomarkers for the evaluation of fish health in the estuarine ecosystem.

2. Material and methods

2.1. Sample collection

Fish were caught with a stow-net fishing vessel (opening area of 135 m2, mesh size of 10 mm at the cod end) at four stations along the Elbe estuary (Fig. 5.1) in the summer period from 25 to 29.08.2021. Sampling stations were chosen within different estuarine sections classified by dominating abiotic drivers (Amann et al., 2012): Station ML-Elbe kilometer 663 within oxygen minimum zone (OMZ) (stream km 620–650, low oxygen, summer <2 mg/L, salinity <0.5 psu), stations SS-Ekm 665 and BB-Ekm-692 within the maximum turbidity zone (MTZ) (stream-km 650–705, high loads of suspended matter, salinity >0.5 < 5 psu) and station MG-Ekm 715 within post-MTZ (Ekm 705–730, transition full marine, salinity >1 < 20 psu). See Fig. 5.1 for sampling overview map and Fig. 5.2 C for abiotic conditions in the water column at sampling times. Sampling procedures followed the standards described in the German Animal Welfare Act (§4 TierSchG). Exemptions to the ordinances on nature reserves were obtained (see Permits). 3102 individuals of age 0+ were caught at the sampling stations (200 at MG, 134 at BB, 208 at SS and 2560 at ML). Six individuals from each ebb haul (n = 24, total length 16 ± 2 cm) were randomly chosen and processed immediately on board. Trends in physiological measures (length, weight, body condition) are representative in the subsets when compared to the larger sample size (see Fig. A.1, (Amieva Mau, 2022)). Physiological and abiotic sample data are supplied in Appendix C. Sample processing was conducted in the following standardized manner: After being brought on board, the fish were stunned with a blow to the head, before being killed with a heart stab. Samples were cooled to 4° until sampling immediately afterwards to ensure best RNA quality. Fish were measured and weighed before recovering gill bacteria with sterile cotton swabs from the middle part of the second and third arch. The same part of the gill tissue as well as the right most edge of the liver was resected for preservation. All samples were kept on dry ice on board and moved to −80 °C until further procession. Tissue samples were first placed in tubes with RNAlater (Macherey-Nagel) for 1 h before they were moved to dry ice. Abiotic conditions (oxygen & salinity levels, Secchi depth, temperature, pH) were measured at start and end of each haul using a multi-probe (Hanna HI 9829 and Secchi disc) at the water surface. Water samples (n = 4) obtained from the water column were vacuum filtered onto 0.2 µm polycarbonate membranes (Whatman™ Nucleopore™ Track-Etch). Whole fish samples were frozen at −20 °C and further analyzed in the lab: body indices were determined (Fulton's body condition (FultonK), hepato-somatic - (HSI) and spleno-somatic indices (SSI) see (Petitjean et al., 2020), stomach content was weighed and age determination from otoliths and scales was performed.

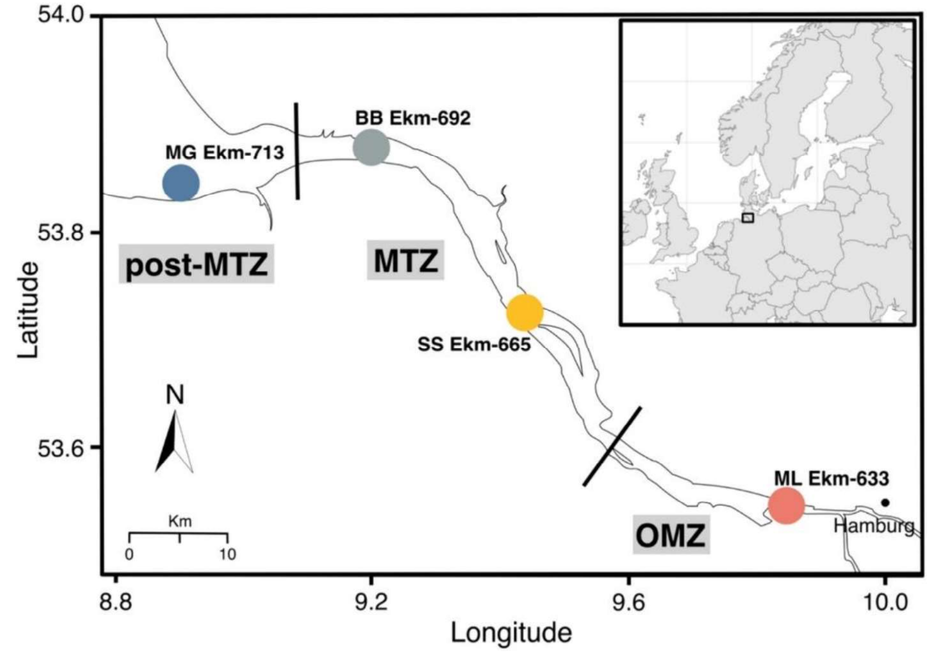


Fig. 5.1. Elbe estuary with section classification following (Amann et al., 2012): Oxygen minimum zone (OMZ), maximum turbidity zone (MTZ), and post-MTZ and sampling locations Mühlenberger Loch (ML), Schwarztonnensand (SS), Brunsbüttel (BB) and Medemgrund (MG) with Elbe river kilometer (Ekm).

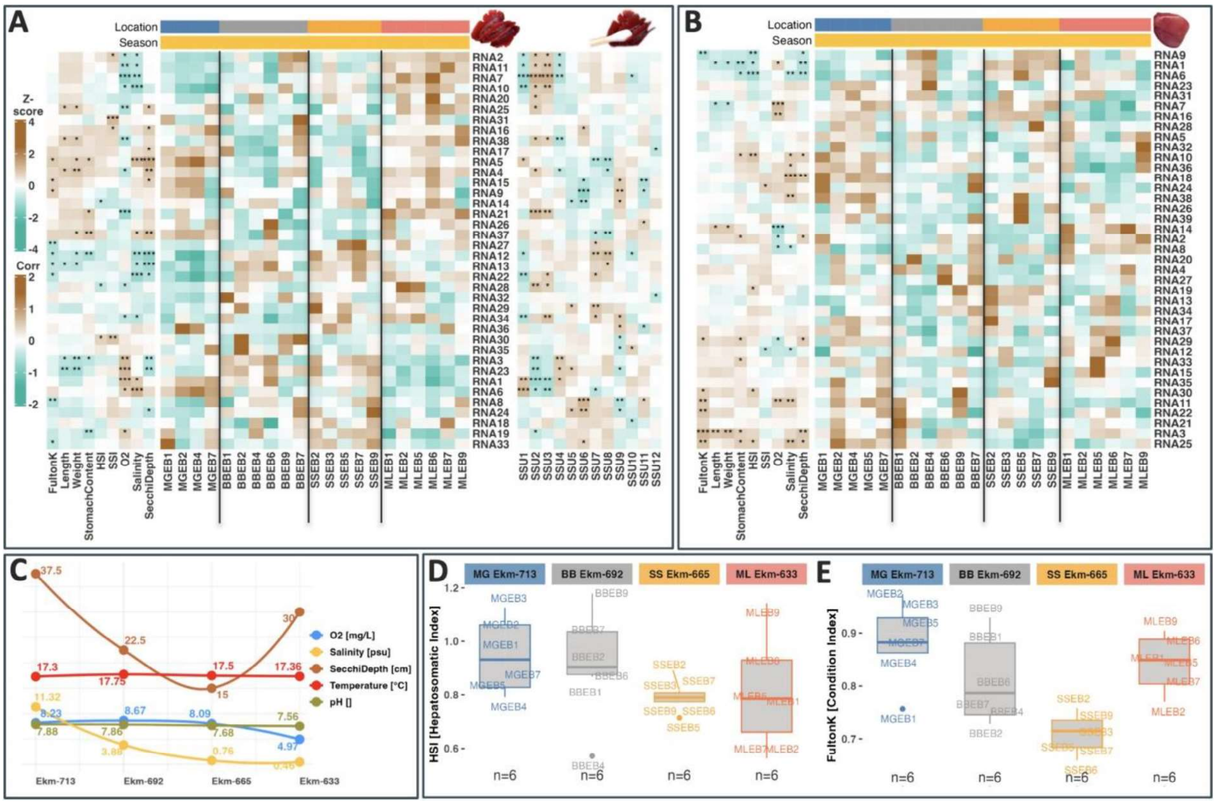


Fig. 5.2. Relationship between gene expression, bacterial composition, biometric fish data and abiotic conditions for Elbe estuarine zander. Integrated heatmap of gill network analysis (A) showing correlation between host gene expression module eigengenes (middle panel: Gill-RNA0-38), physiological traits (left panel: FultonK, StomachContent, HSI (hepatosomatic index)) and relevant abiotic factors (left panel: O2, salinity, Secchi depth) as well as microbiome abundance module eigenASVs (right panel SSU0-12). The middle panel shows Z-score of eigengene values per module as rows for individual fish ordered for

sampling location in upstream direction as columns. The left panel shows Pearson correlation strength between eigengene and host/external traits, the right panel shows Pearson correlation to SSU eigenASVs (brown representing positive, zero white and green negative correlation) and statistical significance indicated by stars: * $p < 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. Integrated heatmap of liver network analysis of matching fish (B) with host module eigengenes (right panel: Liver-RNA0-39) correlated to physiological traits and abiotic factors (left panel). Abiotic factors measured in the water column are depicted for sampling stations (C) as well as selected physiological measurements of HSI (D) and Fulton's condition (E).

2.2. Transcriptome

2.2.1. RNA extraction

Total RNA extraction from gill and liver tissues was performed via sample homogenization (45 s) with ceramic beads in peqGOLD Trifast (Peqlab), RNA dissolving in chloroform and mixing with 70 % ethanol before immobilization on a silica membrane (Crystal RNA Mini Kit, BiolabProducts). On-column DNA digestion with RNase free DNase I (Qiagen) was performed for all samples. RNA quantity and integrity were controlled by spectroscopy (Nanodrop, Thermo Fisher) and on denaturing agarose gels and Bioanalyzer (Agilent Technologies) (avg. RIN 9.6 ± 0.6 over 48 samples). For every individual (n = 24) liver and gill mRNA was isolated using either NEBNext Poly(A) Magnetic Isolation Module (New England BioLabs) followed by stranded cDNA library creation (NEBNext Ultra II Directional RNA Library Preparation kit (New England BioLabs)) or the Illumina RNA stranded mRNA kit including polyA enrichment. Individually barcoded libraries were 2 × 150 paired-end sequenced on NovaSeq6000 (Illumina) (300 cycles, v1.5 reagents, S4 FlowCell) on three different lanes resulting in 3.4 billion reads (avg. ca. 25 ± 6.8 million). To account for possible batch effects from library preparation and sequencing runs, biological replicates from sampling location are spread over runs.

2.2.2. Transcriptome analysis

Transcriptome analysis was performed on the University Hamburg Hummel HPC. Read files were quality controlled using FASTQC (v 0.11.9) and MultiQC (1.12) followed by removal of adapter sequences, clipping of first and last base pairs, quality filtering bases lower Phred score 20 and discarding reads lower than 20 base pairs in length via TrimGalore (v 0.6.10) wrapper of cutadapt (Martin, 2011) and FastQC (Andrews, 2010). Clean reads were mapped to the genome of *Sander lucioperca* SLUC_FBN_1.2 (Nguinkal et al., 2019) using Hisat2 (v.2.2.1) (Kim et al., 2015) (overall mapping rate 99 ± 1 %) followed by expression determination via featureCounts (subread v.2.0.2) (Liao et al., 2014) (assignment rate 51 ± 5.4 %; 18 ± 5 million assignments). All downstream analyses were performed in R (v.4.3.0) using visualization packages ggplot2 (v.3.4.2) and cowplot (v.1.1.1). Genome wide annotation databases org.XX.eg.db (v.3.17.2) (Carlson, 2019) for human, mouse, and zebrafish as well as AnnotationHub (v3.17) (Morgan, 2023) entries for zander were used to translate between different gene identifiers, names and symbols. CDS regions from the reference genome were annotated using diamond (v.2.0.15) blast against UniProt, UniRef90, TrEMBL, RefSeq-Protein and Human EMBL-EBI reference proteome databases. Blast results were filtered for E-value <1e-6 and bit score higher 50, for multiple hits to the same transcript, the one with the lowest E-value was retained. Wherever human genes could be assigned to transcripts on gene-level the human annotation was used for pathway analyses.

2.2.3. DEA & Functional term enrichment

Differential expression analysis was performed to verify network analysis results via DESeq2 (v. 1.41.4) (Love et al., 2014) (significance when FDR adjusted p value <0.05 & Log2FoldChange (LFC) 0.05 or 1) contrasting sampling locations along the estuary. Genes failing to meet expression threshold of transcripts per million mapped reads (TPM) of 1 in at least three samples were excluded from downstream analyses (Lataretu and Hölzer, 2020). Genes were aggregated and hierarchical clustered using in ComplexHeatmap (v.2.17) (Gu et al., 2016). Functional enrichment of heatmap clusters was performed via overrepresentation analyses as described in Section 2.3. For results see Fig. A 2 & A 3.

2.3. Microbiome

2.3.1. DNA extraction

Swabs were incubated in a mix of lysozyme, mutanolysin and lysostaphin before the cells were physically disrupted in a bead mill (Travis et al., 2020). Proteinase K digestion in CTAB buffer was followed by chlorophorm-phenol extraction. Nucleic acids were precipitated in PEG 6000 using LPA-carrier and recovered and washed repeatedly with 95 % and 70 % ethanol before resuspension in water (Neubauer et al., 2021). QC was performed via spectroscopy (Nanodrop, Thermo Fisher) and gel electrophoreses.

2.3.2. DNA sequencing

The V3-V4 variable regions of the 16S rRNA gene were amplified in a one-step PCR using the primer pair 341F-806R (dual-barcoding approach (Kozich et al., 2013); primer sequences: 5′-CCTACGGGAGG-CAGCAG-30 and 5′-GGACTACHVGGGTWTCTAAT-30). After verification of the presence of PCR-products by gel electrophoresis, normalization (Sequal Prep Normalization Plate Kit; Thermo Fisher Scientific, Waltham, USA) and equimolar pooling was performed. Sequencing was conducted on the MiSeq platform (MiSeqFGx; Illumina, San Diego, USA) with v3 chemistry (2 × 300 bp). The settings for demultiplexing were 0 mismatches in the barcode sequences.

2.3.3. DNA bioinformatics

Sequence data trimming, amplicon sequence variant (ASV) prediction and taxonomic identification were performed using cutadapt, FastQC and DADA2 (v.1.29.0) (Callahan et al., 2016). Quality profiles of paired reads were inspected and truncated at 270 and 190 for forward and reverse reads. ASV inference was performed with pooling method and taxonomy assignment used the SILVA SSU v138 taxonomic database (Quast et al., 2013). Accuracy of DNA extraction, sequencing until taxonomic assignment was validated via inclusion of mock community samples (ZYMO research). ASV table and sample data were parsed to phyloseq (v 1.45.0) (Mcmurdie and Holmes, 2012) removing ASVs taxonomically assigned to non-bacteria. Low abundance taxa with sum of counts lower than 0.005 % of total sum of all counts were filtered reducing the number of zeros in the dataset by 50 % to improve interpretability and minimize the risk of spurious correlations. A total of 1285 bacterial ASVs were included in further analyses, minimum sampling depth was ca. 7000, (avg. ca. 21,000 ± 13,000) clean sequences for fish samples. Alpha diversity measures were calculated via vegan package (v. 2.6.4) (Oksanen et al., 2022). The core microbiome was determined from relative abundance data via microbiome package (v. 1.23.0) (Lahti and Shetty, 2017) with filtering detection threshold to 0.0001 % within samples and 90 % prevalence. Centered log-ratio (CLR) transformation to the ASV matrix was applied following best practices for handling of compositional data (Gloor et al., 2017) using the microbiome package (Lahti and Shetty, 2017). The transformed data were used for visualization and network analyses. Permutation multivariate analysis of variance was used to test differences in community structure between fish and bacterioplankton as well as between fish sampling groups in vegan followed by post hoc pairwise t-test via pairwiseAdonis (Martinez Arbizu, 2020). Detailed results in Table A 2. Negative binominal GLM fitting and Wald statistics in DESeq2 (v. 1.41.4) (Love et al., 2014) were used to identify taxa with significantly changing abundances between conditions used to verify the network analysis results. For results see Table A 3 and Fig. A 5 F.

2.4. Holobiont analyses: WGCNA

A Mantel test based on distance matrices was used as an initial approach to reveal overall matrix correlations (Fig. A 4). Weighted gene co-expression network analysis (WGCNA) (v.1.77-1) (Langfelder and Horvath, 2008) was then used for network inference using the following steps and parameters: 1. Signed network construction using biweight midcorrelation measure (Wilcox, 1997). Weighted adjacency is created via soft-thresholding, multiplying the co-expression matrix by a power that leads to approximate scale-free topology. Signed Topological Overlap Measure (TOM) is then used to measure interconnectedness accounting for indirect interactions and assignment of genes and bacterial clusters was performed via hierarchical clustering. Three samples were identified as outliers in both tissue RNAseq datasets by PCA and Euclidean distance clustering and excluded for network analysis and DEA. Parameter list: networkType = "signed", TOMType = "signed", corType = "bicor", minModuleSize = 30/5 (RNA/SSU), reassignThreshold = 0, deepSplit = 3, mergeCutHeight = 0.25. Our parameters were set to allow highest reproducibility between the different datasets in this study and upcoming samplings. Minimum module sizes of 30 and 5 for transcriptomic and SSU (Small subunit rRNA) datasets were chosen to facilitate interpretability. The first principal components of each module (Eigengene/EigenASV) are considered representative for the gene expression or bacteria abundance profile. Functional roles were assigned to the network modules by overrepresentation analyses against the KEGG- (Kanehisa and Goto, 2000) and GO-databases (Aleksander et al., 2023) using ClusterProfiler (v.4.9.0) (Wu et al., 2021). A subset of genes with correlation between gene expression profile and module eigengene (module membership) higher 0.8 was used for function annotation to increase interpretability. From each module, nodes with highest intramodular connectivity from the adjacency (hubs) were inspected as they are considered and shown functionally important (Langfelder et al., 2013). Module eigengenes/ASVs where correlated between the different networks and to physiological traits of the fish (Fulton's body condition, weight, length, stomach content weight, HSI, SSI) and external abiotic factors (DO levels, salinity, Secchi depth) via Pearson correlation. An integrated heatmap analysis approach was used to visualize host responses and holobiont interaction following a workflow developed by Strand et al., (2021).

2.4.1. Principal component analysis

Principal component analyses (PCA) were performed on variance stabilizing transformed counts for RNAseq data or CLR transformed counts for 16S data using PCAexplorer and PCAtools (Blighe, 2023; Marini and Binder, 2019). Evaluation of clustering in PCA-plots indicated no obvious batch effects between sequencing runs. Nevertheless, ComBat-seq (Zhang et al., 2020) batch effect adjustment tool was applied on the datasets but lead to an overall non-batch effect related reduction in variance and was discarded for further analyses.

3. Results

To gain insight into fish response and fish-microbiota interactions under steep abiotic gradients in an estuarine habitat, we applied an integrative approach of tissue specific RNA-seq and gill epithelial mucus 16S rRNA amplicon sequencing collected from juvenile zander from Elbe estuary during late summer. The sampling sites spanned mesohaline to freshwater habitats (Kennish, 2002), with DO levels, turbidity and salinity showing variation between the discreet estuarine areas (Amann et al., 2012): The post-maximum turbidity zone (post-MTZ, station: MG), the maximum turbidity zone (MTZ, stations: SS, BB) as well as the oxygen minimum zone around the Hamburg harbor area (station ML) (Fig. 5.1).

3.1. Network analysis

3.1.1. Post-maximum turbidity zone – salinity adaptation

The post-MTZ was characterized by mesohaline conditions ranging from 9.0 to 14.9 psu and stable DO levels between 8.2 and 8.5 mg/L (see Fig. 5.2 C). Only a few RNAseq modules positively correlated to salinity levels exhibited functional enrichment. Gill-RNA-1 (753 genes with module membership MM > 0.8, Pearson's correlation R = 0.54 and p value 0.01 to salinity levels) was enriched in cellular communication and signaling (focal adhesion, extracellular matrix (ECM)-receptor interaction, cell adhesion), signal transduction (PI3K-AKT, MAPK, cGMP-PKG) and axon guidance (compare Fig. 5.2 A & B & Fig. 5.3 C). Module hub genes were transmembrane protein 204 (tmem204) and protein tyrosine kinase 2 (ptk2aa). Genes in Gill-RNA-5 (144 genes, salinity R = 0.67, p 7.8E-04) were involved in ER protein processing and Gill-RNA-37 (22 genes, salinity R = 0.46, p 3.4E-02) in glutathione metabolism (gpx7, mgst3a). The liver tissue-specific response in RNA-Liver-10 (22 genes, salinity R = 0.49, p 0.022) was related to fatty acid metabolism (elovl5, scdb).

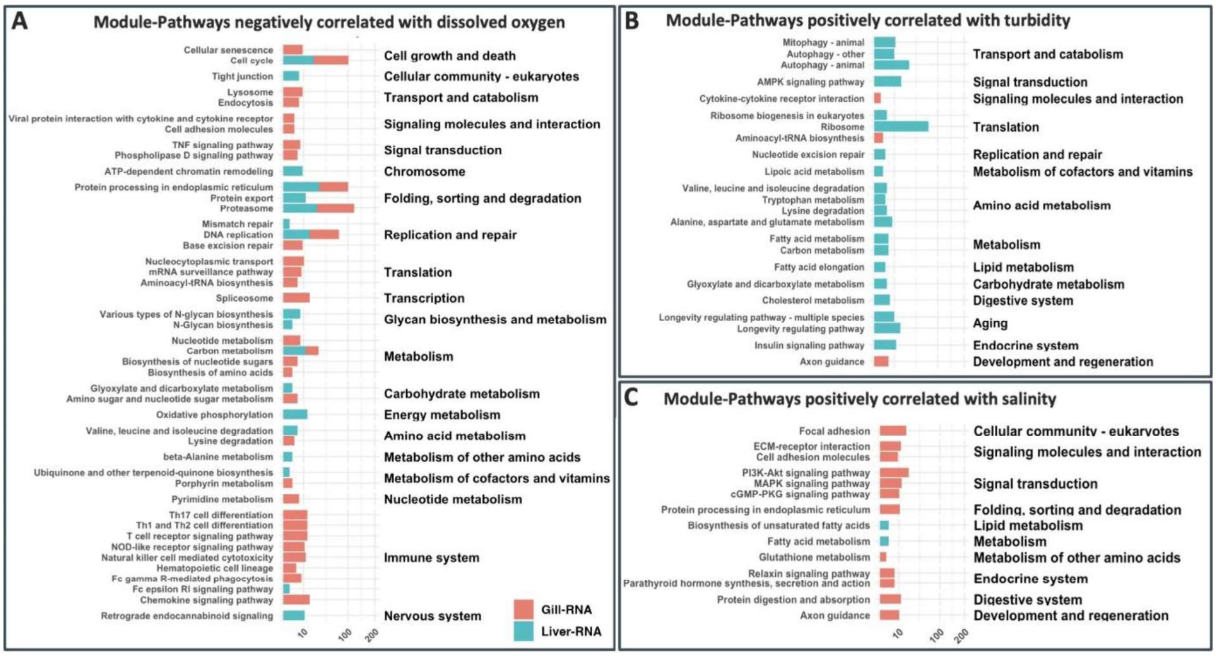


Fig. 5.3. Tissue specific gene regulation in relation to estuarine sections. KEGG pathway enrichments in modules correlated with abiotic traits from tissue specific network analyses are shown. Modules with negative correlation to dissolved oxygen levels (A) and positive correlation to turbidity (B) and salinity (C) are indicative for gene expression patterns within the oxygen minimum zone, the maximum turbidity zone and the post MTZ. In each plot KEGG-level 1 (left y Axis) and KEGG- level 2 (right y Axis) against the number of genes within each module with >0.8 Module membership are depicted.

3.1.2. Maximum turbidity zone – enhanced energy metabolism

The maximum turbidity zone was marked by high particle load (Secchi depth of 10–30 cm), salinity fluctuation between 5 and 0.6 psu (SS: 0.9–0.6 psu, BB: 1.6–5.2 psu) but stable DO levels 7.6–8.8 mg/L (see Fig. 5.2 C). Gill-RNA modules with significant negative correlation to Secchi depth values comprised Gill-RNA-3 (1469 genes, Secchi depth R = −0.55, p 9E-3) enriched in ribosomal genes of both subunits and Gill-RNA-13 (50 genes, Secchi depth R = −0.69, p 5E-4) that contained genes involved in axon guidance (compare Fig. 5.2 A & B & Fig. 5.3 C). Liver-RNA-1 (754 genes, Secchi depth R = −0.57, p 5E-3) comprised genes related to autophagy (AMPK, mTOR, Insulin, FoxO, adipocytokine), mitophagy and peroxisome with autophagy related hub gene bnip4. Liver-RNA-6 (118 genes, Secchi depth R = −0.56, p 6.6E-3) was composed of ribosomal subunit genes. Liver-RNA-9 (138 genes, Secchi depth R = −0.45, p 3.6E-2) was highly enriched in metabolic pathways comprising fatty and amino acid degradation (tryptophan metabolism, valine, leucine and isoleucine degradation, lysine degradation, alanine, aspartate and glutamate metabolism).

3.1.3. Oxygen minimum zone – cellular stress and immune reactions

The oxygen minimum zone was characterized by low DO levels between 4.6 and 5.0 mg/L (46–52 % saturation) and stable salinity levels (0.46 psu). Seven Gill-RNA modules were negatively correlated to oxygen levels in the surrounding milieu, five of them were also negatively correlated to salinity (see Fig. 5.2 A & B). Gill-RNA-Module 2 (455 genes, DO R = −0.52, p 0.015) was highly enriched in replication and repair pathways (DNA replication, base excision repair, mismatch repair, nucleotide excision repair), cell growth and death (cell cycle, apoptosis, necroptosis), folding, sorting and degradation (proteasome), immune system (antigen processing and presentation) as well as signal transduction (NF-kappa B signaling pathway). Hub genes comprised proteasomal subunits (psmb9a, psma6l, psmb10, psme1), innate immunity related irf1b and erap2. Gill-RNA-4 (326 genes, DO R = −0.51, p 0.017) showed enrichment in protein procession (proteasome, protein processing in ER, Protein export), genetic information processing (nucleocytoplasmic transport and spliceosome). Hub genes were psmd2, ups5, usp14 involved in protein processing as well as cell proliferation related ppp5c. The immune response in gill tissue was divided between two modules. Gill-RNA-7 (123 genes, DO R = −0.79, p 2.6e-05) included innate immune response parts (chemokine signaling and viral protein interaction with chemokine). Hub genes were microbicidal oxidase system of phagocytes (cyba, nox1) and MHC class II paralogues. Three variants encoding for eosinophil peroxidase (epx-like) and mucin-2 like isoforms were most correlated to DO levels (R-0.85) and highly upregulated in the Hamburg area (LFC > 1, see Fig. A 2). Gill-RNA-11 (213 genes, DO R = −0.59, p 4.5e-03) was enriched in adaptive immune system processes specifically T cell receptor signaling, T-helper cell differentiation, NK cell mediated cytotoxicity and general chemokine signaling. Hub genes include the protein tyrosine phosphatase ptprc and lymphocyte cytosolic protein 2 lcp2a. Gill-RNA-21 (64 genes, DO R = −0.77, p 4.6e-05) was enriched in porphyrin metabolism (hubs: rhd and alas2) and strongly upregulated hemoglobin subunit alpha-2-like and beta-2-like. Gill-RNA-25 (19 genes, DO R = −0.6, p 4e-03) shows enrichment in lysine degradation (kmt2d, ezh2, kmt2bb). The liver specific response was less pronounced compared to gill, Liver-RNA-2 (2327 genes, DO R = −0.45, p 0.03) was enriched in genetic information processing pathways (proteasome, protein processing in ER, DNA replication, spliceosome, base excision repair), cellular processes (cell cycle) and metabolism (carbon metabolism, N-glycan biosynthesis, TCA cycle). Hub genes include again usp14 and ppp5c. Liver-RNA-8 (81 genes, DO R = −0.49, p 0.02) was enriched in energy metabolism (oxidative phosphorylation), metabolism (of amino acids, cofactors, vitamins), mismatch repair as well as protein procession (proteasome, protein export, N-glycan biosynthesis).

3.2. Gill microbial community structure

Approximately 630,000 trimmed, filtered and merged reads across 24 zander and four water samples resulted in 42.000 ASVs. Filtering for ASVs contributing at least 0,005 % of overall abundance reduced sparsity in the dataset by 50 % and retained 1285 ASVs for further analysis (complete ASV list in Appendix B). Species accumulation analysis indicated that the rare microbiome was not sufficiently captured in the dataset (see Fig. A 5 A) but overall composition was when excluding rare taxa (Fig. A 5 B).

3.2.1. Host-associated differ from free-living bacterial communities

We identified seven bacterial phyla associated with the fish microbiome and eight phyla in the surrounding water column (Table A 1). There was a significant difference between the microbiome of the fish and that of the surrounding water column (DF 1, F 7.5686, p 0.001) (Fig. A 5C, Table A 2). Of the 1285 ASVs identified 51 were found only in the water column while 600 were unique to the fish gill microbiome (Fig. 5.4 B). The water column was characterized by a large abundance of Actinobacteriota (25 % in water samples, 1–6 % in fish), Proteobacteria (38 %, fish: 53–78 %), Bacteroidota (15 %, fish: 14–32 %), Verrucomicrobiota (8 %, fish: 1–3 %) and several phyla not represented in fish samples at all: Cyanobacteria (4 %), Nitrospirota (3 %), Acidobacteriota (2 %), Planctomycetota (1 %). Firmicutes, Deinococcota and Desulfobacterota in the contrary were only recovered from fish gills.

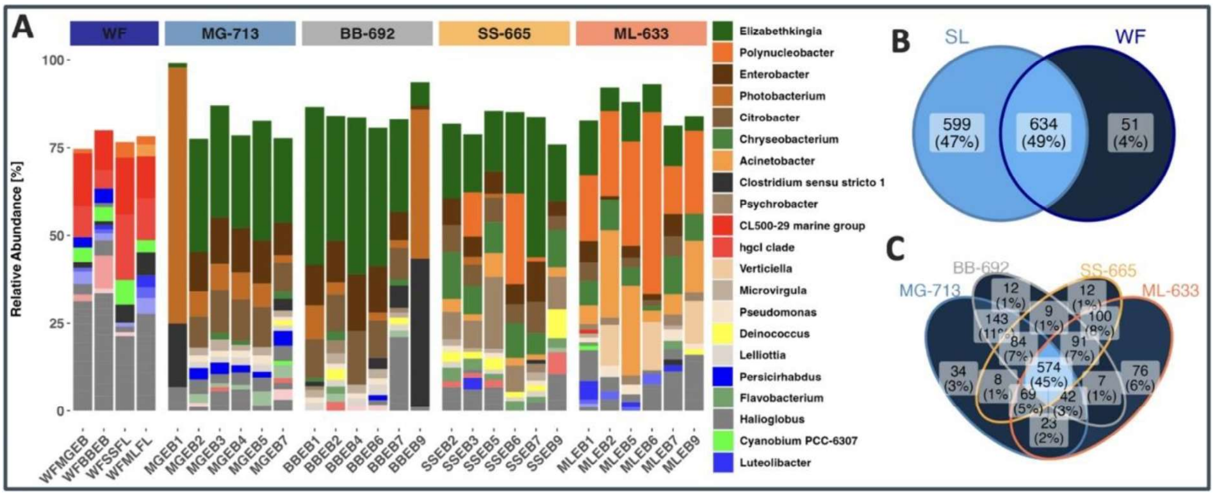


Fig. 5.4. Bacterial composition fish gill mucus and bacterioplankton. Compositional barplot (A) shows genus accumulated relative abundance (>1 %) per sample. Venn Diagrams depict presence/absence of bacterial ASVs found in water samples (WF) and on fish (SL) (B), and in fish swabs along the curse of the estuary (C).

3.2.2. Host-associated communities diverge along the course of the estuary

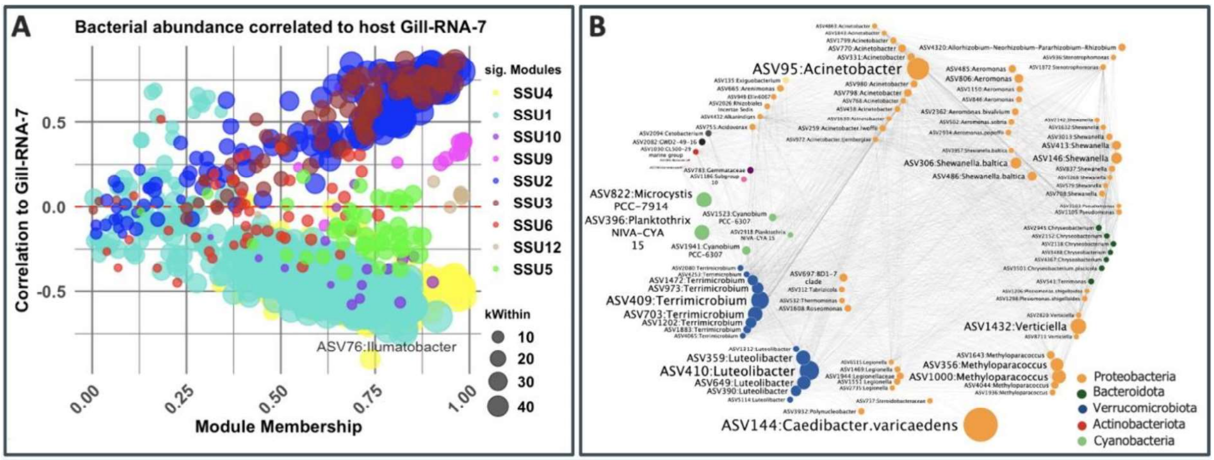
We found a significant site-dependent difference in the bacterial community composition of the fish gill (DF 3, F 3.5533, p 0.001) between all sampling locations (Fig. A 5 C, PC2, Table A 2). 45 % of ASVs occurred in at least one fish from each sampling site and there were few site-specific taxa (1–6 %) (Fig. 5.4 C). The community was dominated by Proteobacteria followed by Bacteroidota and Actinobacteria which were the only phyla present on all individuals. The ratio of abundances between Proteobacteria and Bacteroidota was subjected to strong changes (MG: 62/21 %, BB: 53/20 %, SS: 54/32 %, ML: 78/14 %). Enterobacterales (32 %), Flavobacteriales (19 %), Burkholderiales (13 %), Pseudomonadales (9 %) and Clostridiales (6 %) make up 80 % of the relative bacterial abundance over the estuary. Enterobacterales (49 % in most downstream – 12 % in most upstream sampling station) steadily decrease over the course of the estuary with *Photobacterium* (27 % – 0 %), *Citrobacter* (8–3 %), *Enterobacter* (7–2.5 %), *Lelliottia* (4–2 %) being the most abundant genera. Burkholderiales (3–36 %) with the genera *Polynucleobacter* (0–28 %) *Verticiella* (0–7 %) and the *Pseudomonadales* (5–17 %) with the genera *Acinetobacter* (0–14 %) and *Psychrobacter* (0–2 %, SS 8 %) show increasing relative abundance in fish gill swabs in upstream direction. Flavobacteriales show differing patterns with the genera *Elizabethkingia* (16–8 %) steeply decreasing over estuary and the genus *Chryseobacterium* (0–6 %, SS 9 %) increasing with a peak in the middle of the estuary. Alpha diversity slightly changes along the course of the estuary, observed richness steadily increased from 364 to 502 ASVs (sampling group means) in upstream direction with slight changes in Shannon index: MG: 3.9; BB: 3,6; SS: 4.3; ML: 4.0.

3.2.3. The core microbiome community of estuarine zander gills

The core microbiome comprised 43 ASVs from 11 orders predominated by roughly even relative abundance of Proteobacteria and Bacteroidota (Fig. A 5 D & E): Proteobacteria (Enterobacterales (18 %), Pseudomonadales (1 %), Burkholderiales (1 %), Caulobacterales, Sphingomonadales), Bacteroidota (Flavobacteriales (16 %), Chitinophagales (1 %), Sphingobacteriales), Actinobacteriota (Microtrichales (1 %), Corynebacteriales, Micrococcales), Deinococcota (Deinococcales) & Verrucomicrobiota (Chthoniobacterales). While the relative proportion of core taxa in the total microbiome declined along the course of the estuary (56 to 28 %), the relative proportions within core taxa stayed overall stable (Bacteroidota/Proteobacteria from 55/40 to 60/35 %). The Hamburg harbor area has the largest impact on the core microbiome, with slight increases in Bacteroidota. CL500-29 marine group was the only taxon shared in large amounts (>1 %) between core fish gill microbiome and surrounding water column.

3.2.4. Potential pathogens correlate to host immune response

The bacterial network modules Gill-SSU-2 (Fig. 5.5 B) and 3 were correlated with the host gill immune modules (Gill-RNA-7 & 11) (Fig. 5.5 A and Fig. A 6), comprising 17 genera with Pearson correlation (>0.6) and intramodular connectivity (>0.8) (Fig. 5.5 C). Of these *Verticiella* (4 ASVs), *Shewanella* spp., *S. baltica* and *S. putrefaciens* (9 ASVs), *Aeromonas* (8 ASVs), *Acinetobacter* spp., *A. johnsonii*, *A. tjernbergiae*, *A. iwoffii* (24 ASVs), *Polynucleobacter* (4 ASVs), *Plesiomonas* (2 ASVs) and *Chryseobacterium* spp., C. *piscicola*, *C. haifense* (13 ASVs) were with almost all strains absent from the bacterioplankton. In the contrary, Methylococcaceae (4 ASVs), Gemmataceae (4 ASVs), *Luteolibacter* (5 ASVs), *Terrimicrobium* (9 ASVs), *Cyanobium* (3 ASVs) and *Legionella* (7 ASVs) were abundant in both water and fish samples.



C

Phylum	Order	Lowest Taxonomic level	Relative Abundance [%]	SSU Moddule	Module Membership	Correlation to Gill-RNA-7	Correlation to Gill-RNA-11
Proteobacteria	Enterobacterales	ASV306: Shewanella.baltica	Avg 0.05; max 0.53	2	0,90	0.85	0.61
		ASV413: Shewanella	0.04; 0.42	2	0.98	0.80	0.50
		ASV415: Shewanella	0.07; 0.44	3	0.89	0.78	0.60
		ASV221: Shewanella putrefaciens	0.03; 0.36	3	0.85	0.72	0.69
		ASV485: Aeromonas	0.02; 0.16	2	0.80	0.78	0.56
		ASV158: Aeromonas	0.07; 0.76	3	0.83	0.73	0.59
		ASV846: Aeromonas	0.01; 0.03	2	0.82	0.63	0.63
		ASV1298: Plesiomonas shigelloides	0.01; 0.08	2	0.91	0.69	
	Pseudomonadales	ASV95: Acinetobacter	0.15; 1.09	2	0.90	0.75	0.50
		ASV972: Acinetobacter.tjernbergiae	0.01; 0.09	2	0.86	0.74	
		ASV259: Acinetobacter.lwoffii	0.02; 0.14	2	0.89	0.69	
		ASV977: Acinetobacter.johnsonii	0.02; 0.17	3	0.91	0.77	0.50
		ASV1105: Pseudomonas	0.01; 0.10	2	0.86	0.74	
		ASV4432: Alkanindiges	0.01; 0.10	2	0.86	0.69	
	Xanthomonadales	ASV1872: Stenotrophomonas	0.01; 0,17	2	0.84	0.70	
	Methylococcales	ASV1000: Methyloparacoccus	0.08; 0.61	2	0.83	0.79	
		ASV272: Methylococcaceae	0.30; 1.11	3	0.96	0.87	
	Legionellales	ASV1944: Legionellaceae	0.02; 0.08	2	0.89	0.76	
	Burkholderiales	ASV15: Verticiella	1.14; 11.51	3	0.83	0.86	
		ASV25: Polynucleobacter	3.02; 38.54	3	0.84	0.77	0.64
Verrucomicrobiota	Chthoniobacterales	ASV973:Terrimicrobium	0.06; 0.42	2	0.90	0.84	0.63
Bacteroidota	Flavobacteriales	ASV3488: Chryseobacterium	0.01; 0.08	2	0.80	0.68	
		ASV3501: Chryseobacterium.piscicola	0.01; 0.04	2	0.87	0.64	0.58
Planctomycetota	Gemmatales	ASV140: Gemmataceae	0.15; 0.71	3	0.95	0.84	0.53

Fig. 5.5. Specific bacterial strains highly correlated with innate and adaptive immune response in host gill. Bacterial abundance correlated to host gill innate immune response Gill-RNA-7 module eigengene (A). ASV module membership on X-axis and Pearson correlation on Y-Axis, point size indicates intramodular connectivity, color core depicts module membership. The co-occurrence network module Gill-SSU-2 (B) highly correlated with host immune response is shown where point size indicates average relative abundance ASVs with highest correlation to host immune response modules Gill-RNA-7 and 11 (C). Relative abundance values over all samples (Avg.) and for the highest sample (max.) are shown. Only the first strain per lowest taxonomic level with Module Membership >0.8 and Pearson correlation >0.6 is depicted.

4. Discussion

In this study we sought to understand, through OMICs, how the predatory fish zander responds to changing abiotic and biotic (microbiome) conditions along an estuary, as a means for understanding existing and future paradigms of environmental change and fish biomass losses. Here we were able, using a global molecular approach, to demonstrate how fish physiology is impacted in a tissue-specific manner, both by different environmental impacts (salinity, turbidity and oxygen availability) and by changes in the relative contribution of core and potentially pathogenic bacteria on the fish gills.

4.1. Salinity adaptation

While zander larvae are stenohaline bound to freshwater and oligohaline areas, juveniles acquire the capacity to adapt to a wider range of osmotic fluctuations. Osmoregulation requires synergistic action of a complex sensing and signal transduction network that could be achieved by enhanced osmosensing in the juvenile zander via mitogen-activated protein kinase (MAPK) pathway (Kültz and Avila, 2001) and PI3k-Akt regulating involved salinity stress response (Zhao et al., 2021). Further, we found cell-cell/ECM interactions related to gill remodeling processes during salinity fluctuations (Jeffries et al., 2019; Lam et al., 2014; Zhou et al., 2022) and protein tyrosine kinase 2 (here ptk2aa) as hub gene in gill remodeling (Zhang et al., 2023). Major epithelial cells in fish gills, pavement and mitochondria-rich cells, play complementary roles in ion transport (Evans et al., 2005). Their growth and differentiation may be organized by axonal guidance contributing to functional plasticity in estuarine fish (Lai et al., 2015). In accordance, axonal guidance was enriched in zander gill from mesohaline till oligohaline areas. While osmoregulation is among the most energy-intensive metabolic activities in teleosts (Bœuf and Payan, 2001), mesohaline zander in this study showed highest biometric values and gene expression pattern related to hepatic fat accumulation (Perera et al., 2020).

4.2. Starvation in murky waters

The mid estuary maximum turbidity zone is marked by liver specific elevated metabolic pathways, autophagy and regulation of ribosomal genes. These correlate with lowest biometric data (length, weight, HSI, and compromised body condition) indicative for nutritional stress (Echevarría et al., 1997). During periods of nutrient deprivation, fish exhibit shifts in energy utilization, depleting liver glycogen (De Pedro et al., 2003; Yang et al., 2019) and relying on lipids as major energy source (Bar, 2014). Lipid droplets are broken down to free fatty acids via lysosomal-autophagy pathway (Wang et al., 2018). In mammalian cells, starvation causes disruption of mitochondrial structure (Domenicali et al., 2001) thus increases ROS and induces autophagy via AMPK pathway (Li et al., 2013). Likewise, fish show increased ROS (Varju et al., 2018) and induced autophagy and mitophagy (Pan et al., 2022). Autophagy is a key pathway mediating damage control and metabolic adaptation (Kroemer et al., 2010) triggered i.a. by hypoxic conditions involving BNIP3 and Beclin1 signaling while nutrient depletion activates AMPK-dependent ULK1 and Beclin1 (Kim et al., 2022) in mammals. Zebrafish ortholog genes include hypoxia-induced bnip3a, closest to human BNIP3, and hypoxia-independent bnip3 and bnip4 (Feng et al., 2011). Here we identified bnip4 and sirt5-like as hub genes related to autophagy. Sirtulin enzymes, such as mitochondria-specific SIRT5, play pivotal role controlling lipid metabolism, mitophagy and apoptosis possibly via AMPK pathway (Guedouari et al., 2017; Hong et al., 2020). These could be indicative for nutrient depletion driven autophagy activation in zander liver.

The upregulation of ribosomal genes appears seemingly counterintuitive as ribosome biogenesis is most energy-consuming (Kraft et al., 2008) and downregulated in starving fish (Salem et al., 2007). However, severely reduced translation might be detrimental for acclimatization responses (Zhu and Dai, 2019), especially in a multi-stress real-world setting. Ribosomes occupy large amounts of amino acids that can be tapped by autophagy during starvation delivering fuel for proteome remodeling (Rabinowitz and White, 2010). However, autophagic tissue degradation has to be balanced by macromolecule synthesis to maintain homeostasis (Rabinowitz and White, 2010).

The cause of the starvation situation remains unclear. Turbidity, in general exerting negative effects on prey capture (Ortega et al., 2020), did not affect foraging success in juvenile zander across a range of turbidity levels (Ljunggren and Sandström, 2007). We did not investigate potential influences on prey availability or altered food spectra, observed in other estuarine predatory fish during increased turbidity (Lowe et al., 2015). As tissue fat content is a crucial factor for overwinter survival of juvenile zander (Rennert et al., 2005), the low weight, HSI and body condition particularly detected in the upstream part of the turbidity zone together with fat and other energy source degradation pathways activation can be expected to affect survival rates of MTZ zander. Sediment load was shown to damage gill tissue in different species (Hess et al., 2015; Lowe et al., 2015), however an anticipated immune response linked to turbidity was not evident here. The relationship between starvation and immune regulation is complex and might relate to suppression of tissue (Salem et al., 2007) and external mucus immune functions (Soltanian and Gholamhosseini, 2019). We cannot rule out starvation related immunosuppression masking underlying processes.

4.3. Cellular stress in oxygen minimum zone

Gills are the primary organ for physiological exchanges and first response to stressors like hypoxia (Evans et al., 2005). Low DO acclimatization involves optimizing uptake and distribution, reflected in enhanced erythrocyte and hemoglobin concentrations (Richards et al., 2009). Identified genes, including hemoglobin subunit beta-2-like hbb2, linked to oxygen transfer and upregulated in OMZ, mirror findings in other percid species like Elbe estuarine ruffe and yellow croaker (Mu et al., 2020; Tiedke et al., 2015). Hypoxic conditions also induce epigenetic modulation by KMT2 family genes (Tsai and Wu, 2014), proposed as adaptive coping mechanism in rainbow trout (Léger et al., 2021) and identified here as module hubs (kmt2d and kmt2bb).

The upregulation of carbon and amino acid metabolism pathways together with TCA and OXPHOS in juvenile zander liver from the estuarine OMZ indicates a locally enhanced energy demand. Remodeling of energy metabolism is an effective strategy in fish to compensate increased demand during hypoxic conditions (Ding et al., 2020) or infection (Xiong et al., 2022). The liver plays a pivotal role controlling energy reserves utilizing amino acids as primary fuel (Jia et al., 2021) with upregulated metabolism (Gracey et al., 2001; Ni et al., 2014) observed during hypoxic episodes. Prolonged hypoxia can increase dominance of aerobic TCA and OXPHOS (Ding et al., 2020).

Local response was further marked by different aspects of cellular stress response (Kültz, 2005) including cell cycle arrest and DNA repair accompanied by affected proteostasis in both tissues. Halting energy-intensive genome duplication processes leverages time for macromolecular repair and allows for energy redirection (Kültz, 2020). Recovery from stressors on the other hand is accompanied by sustained repair processes (Mustafa et al., 2015; Zhang et al., 2021). In our study, ppp5c is a cross-tissue hub gene involved in cell cycle arrest and DNA damage repair (Hinds and Sánchez, 2008) shown dysregulated in fish under severe stress (Bertucci et al., 2020). The ATP-dependent ubiquitin-proteasome system (UPS) mitigates proteotoxicity from damaged proteins (Bard et al., 2018) resulting i.a. from disrupted endoplasmic reticulum (ER) (Hetz, 2012). In both tissues we found moderate hypoxia correlated with UPS and endoplasmic reticulum (ER) stress response (Hwang and Qi, 2018) characterized by luminal chaperone and ER-associated degradation (ERAD) activation. Besides ubiquitin-proteasome subunits, deubiquitinase genes (DUBs) usp5 & usp14 showed highest intramodular connectivity linking enhanced protein turnover with DNA repair (Nakajima et al., 2014), immune response (Jia et al., 2020) and autophagic processes (Wang et al., 2021). Enrichment in apoptosis and necrosis pathways specific in gill indicates stress exceeding cellular tolerance levels (Kültz, 2005) in the exposed tissue.

Adaptive modification of the ubiquitin-proteasome composition by disassembly into the 20S unit increases recognition capacity for oxidatively damaged proteins (Grune et al., 2011). Inflammation as well as oxidative stress induce transcriptional upregulation of PSMB8, PSMB9, PSMB10, encoding specialized beta subunits in the 20S proteasome core creating the so-called immunoproteasome (Früh et al., 1994). Immunoproteasomes are central to processing antigenic peptides presented by major histocompatibility complex class (MHC) I molecules, clearance of oxidized proteins and protection of cells from inflammation induced damage (Johnston-Carey et al., 2016). In mammals, transcription of the inducible B-type subunits is suspected to be controlled by the Interferon regulation factor-1 (IRF-1) signal transduction pathway (Foss and Prydz, 1999). In line, we identified B-type subunits (psmb8a & b, psmb9a, psmb10) together with irf1b as hub genes in the largest oxygen correlated gill module. So far there is relatively little research focusing in depth on proteasomal gene expression in fish. Enhanced expression of psmb (8, 9, 9-L, 10) was shown during antibacterial and antiviral response (Kasthuri et al., 2014) used as discriminative markers for infection monitoring (Miller et al., 2017). The expression and high intramodular connectivity of a full set of 20S proteasome, proteasome activator and inducible immunoproteasome indicate elevation of oxidized proteins due to oxygen stress and elevated ROS-levels and increased activity of immunologically active cells in the gill tissue of Hamburg harbor fish coordinated by irf1b signaling.

4.3.1. Gill immune response

Both, the co-expression networks and the differential gene expression analyses identified specific marker genes for T and B cell signaling upregulated in Hamburg area. Adaptive immune module hubs ptprc as essential regulator of T and B cell antigen receptor (Barashdi, 2021) and T cell signaling related LCP2 (lcp2a) were also identified as immune hubs in bacteria challenged flounder (Wang et al., 2022). The latter was also found regulated upon infection with eukaryotic parasites (Syahputra et al., 2018) and bacteria (Li et al., 2018) indicating cross-species and cross-tissue importance in teleost immune response. Immune cells use reactive oxygen species (ROS) for destruction of pathogen cells involving CYBA and NOX2 encoded phagocyte NADPH oxidase multiprotein complex (Bedard and Krause, 2007). In mucosal immunity NOX1 is hypothesized to replace NOX2 (Geiszt et al., 2003), activity of which requires CYBA, NOXO1 and NOXA1 stimulated by IFN-γ (Kuwano et al., 2006). Phagocyte NADPH oxidase has key regulatory function in innate immune response via ROS mediated signaling in mammals (Thomas, 2018), matching identification of this gene set as innate immune module hubs in juvenile zander. The highly upregulated eosinophil peroxidase variants in Hamburg area indicate involvement of eosinophils in anti-microbial immune response (Mickenberg et al., 2014). Eosinophils are associated with parasitic infections, controlling inflammation and maintaining epithelial barrier

(Gleich, 2000) but have only been studied in a limited number of teleosts including zebrafish (Balla et al., 2010), turbot (Noia et al., 2021) and flounder (Choi et al., 2021). All variants show a strong negative correlation with DO, indicative for eukaryotic infestation in the OMZ. Strong upregulation of Mucin-2 like isoforms involved in GIALT physical barrier in Hamburg area further support the local immune response. These variants have already been found enhanced in skin mucus of salmonids under physiological stress going along with immune suppression and overgrowth in bacteria (Tacchi et al., 2015).

4.4. The zander gill microbiome

Juvenile zander host a complex gill microbiome distinct from that of the surrounding water column which was clearly impacted by the sampling location along the estuary. Although influenced by the bacterioplankton (Pratte et al., 2018), gill structure and function support a unique and highly diverse bacterial composition in wild fish (Minich et al., 2020). Differing tolerances of inhabiting taxa to physiochemical gradients influence the composition of fish mucus communities (Lokesh and Kiron, 2016). The strong variation in bacterial composition along the estuary identified in this study is accompanied by only a slight increase in observed taxa with decreasing salinity. The significant differentiation between sampling sites combined with relatively high similarity of bacterial composition on individuals from the same location might be indicative for low movement patterns of juvenile zander within the course of the estuary. Overall, the oxygen minimum zone and freshwater transition in the Hamburg harbor area were found most influential for the variable composition as well as for the core microbiome. This is indicative for a microbial reorganization and possible disease in these animals (Sehnal et al., 2021). On the one hand the core species, assumed to serve beneficial roles in the host, decline: *Asinibacterium* and *Enterobacter* taxa are considered to inhibit pathogen growth (Halet et al., 2007; Schubiger et al., 2015), *Lelliottia* is described as a critical part in juvenile percides gut microbiomes (Gallo et al., 2023). *Elizabethkingia* is a well-known component in mucus communities in freshwater and in marine fish (Jacobs and Chenia, 2011; Zhang et al., 2022). On the other hand, with the emergence of known freshwater taxa like *Polynucleobacter* (Jezbera et al., 2011) and *Verticiella*, a variety of opportunistic pathogenic taxa gain abundance in the OMZ.

4.4.1. The zander holobiont in the Elbe estuary

Host-microbe and microbe-microbe interaction are gaining more and more attention due to their tremendous importance for fish health (Kelly and Salinas, 2017). A few recent studies focused on the bacterial composition on gills in wild fish (Pratte et al., 2018) assessing pathogen load from the presence of specific genera and species (Itay et al., 2022; Minich et al., 2020). Here, we aimed to describe the holobiont incorporating bacterial abundance with tissue specific gene expression patterns in the host. Stressful conditions are expected to affect the interplay (Yu et al., 2021), oxygen deficiency in aquaculture for example has been linked to immunosuppressive effects (Abdel-Tawwab et al., 2015; Gallage et al., 2017) and increased abundance of potentially pathogenic taxa (Boutin et al., 2013). As such, a substantial number of suspected or confirmed fish pathogens on zander gill aligned with an intensified host immune response in the oxygen minimum zone. Taxa with suspected opportunistic pathogenic functions comprise *Pseudomonas* (Duman et al., 2021), *Chryseobacterium* (Loch and Faisal, 2015), *Acinetobacter* (Kozińska et al., 2014), *Psychrobacter* (Hisar et al., 2002) and *Aeromonas* (8 ASVs), the latter known for causing multi-tissue damage in freshwater fish (Nielsen et al., 2001). Kidney specific transcriptome studies indicated OXPHOS and proteasome to be strongly activated upon *Aeromonas* infection followed by cellular senescence and apoptosis pathways (Xiong et al., 2022) matching our results. *Shewanella* strains (16 ASVs), including the species *S. putrefaciens* and *S. baltica*, showed highest correlation to the immune response and abundances of up to 3.5 % in fish while being scarce in bacterioplankton. *Shewanella* is known for its role in decomposing organic matter under hypoxic conditions (Nealson and Scott, 2006) and various strains have been found in gill mucus communities of marine fish (Itay et al., 2022; Pratte et al., 2018) some of which resemble potentially pathogenic species (Minich et al., 2020). Besides repeated detection in diseased fish (Cocchi et al., 2018; Paździor et al., 2023; Rusev et al., 2016), a recent study found high pathogenicity of *S. putrefaciens* strains with infection rates peaking when the DO values fall below 5 mg/L (Esteve et al., 2017).

The oxygen minimum zone in the Elbe estuary shows persistent DO levels below 5 mg/L during the summer month. Nevertheless, the largest numbers of juvenile zander hatching in freshwater or oligohaline waters reside in this region. The observed cellular stress response, the immune response, the impaired core microbiome and high levels of potentially pathogenic bacteria are not directly correlated with compromised biometrics, but may indicate a burden on the health situation of the fish that precedes these physiological markers. The effects shown here are also likely to have impact on other fish species that hatch and develop in this region and for which a decline in biomass has already been observed in the Elbe estuary (Scholle and Schuchardt, 2020). Immediately downstream from the OMZ, zander show signs of starvation and reduced biometric data. Migration to the lower parts of the estuary, where high biometric values were observed, would require crossing of the OMZ and MTZ. The prevailing conditions could represent a migration barrier due to avoidance behavior (Roberts et al., 2009; Thiel et al., 1995) and thus weaken parts of the population. This would affect the nutrient flow and carbon sequestration in the estuary which is strongly influenced by the presence of top predatory fish species (Atwood et al., 2015; McLean et al., 2019).

Although we focused on the responses to the three dominant environmental factors, these must be regarded as proxies for an environment with numerous biotic and abiotic pressures. The Elbe estuary has a long history of organic compound and heavy metal pollution with toxicity values expected to affect fish communities especially in the harbor area (Wetzel et al., 2013) that were not measured here. Contaminant and pathogen defense both affect the same physiological traits (Petitjean et al., 2019) and stimulate oxidative stress response that was already triggered by the lowered oxygen levels in the OMZ interacting with the overall response of zander from this region.

With this exploratory study, we were able to create a global overview of potential drivers of fish health in this ecosystem and interpret these against the background of experimental data from the literature. With the relatively large amount of high-quality data and independent bioinformatic approaches, we were able to identify patterns based on high numbers of correlated gene regulations. Many findings are consistent with laboratory experiments (e.g. gill remodeling processes in response to salinity and lowered DO values), but for some correlations, such as the interaction of opportunistic bacteria, environmental stressors and the immune response in the host, the study is limited and more in-depth investigations will be required. Especially in the light of recent research findings related to deoxygenation and suppression of the adaptive immune components in fish (Leeuwis et al., 2024), our findings here will need to be validated in large-scale multi-stress experiments across an extended range of methods.

5. Conclusion

This explorative study is one of the first using an unsupervised analysis approach of the microbiome in combination with tissue-specific gene expression in an estuarine wild-fish population. Our results provide deeper insight into prevailing stressors than biometric and abundance data alone, improve the ability to assess the health of fish stocks and to understand population declines. Our findings (Fig. 5.6) reveal that juvenile zander gene expression is significantly impacted by turbidity, related to signs of starvation, and reduced oxygen levels triggering robust immunological and cellular stress responses. The disruption of the core microbiome and the rise of potential pathogens under low-oxygen conditions highlight the pressing need to understand and address the responses of the holobiont. As oxygen deficiency events are projected to increase due to climate change, this study underscores the potential escalation of disease risks, such as shewanellosis. Mowing forward, future studies should consider multi-stress experiments in conjunction with OMICS-based time-series monitoring across additional, likely more vulnerable life stages to validate these results and anticipate how fish populations in highly productive estuarine nursery areas will develop under climate change.

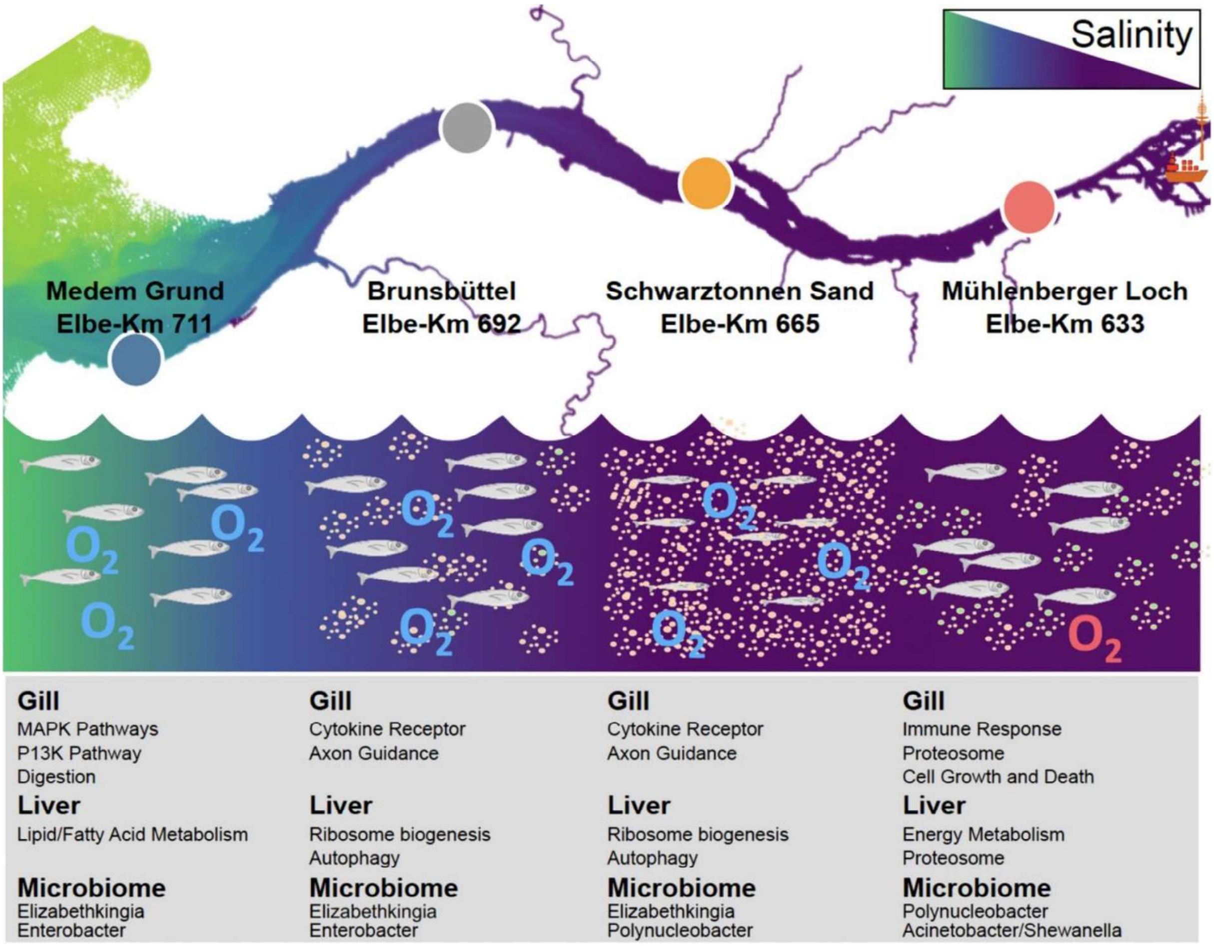


Fig. 6. Graphical summary. The main tissue specific gene expression pattern and most important gill mucus bacteria are shown in the course of the estuary correlated with the dominant environmental drivers (salinity, turbidity, oxygen levels) used as proxies for multiple stressors.

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8. Data availability

Sequence data are deposited in the ENA Sequence Read archive under study PRJEB71116. We made the full analysis available in stepwise R markdown files and illustrated HTMLs, including metadata and supplementary lists under https://github.com/vollkorrn/ElbeEstuarineZander.

9. Permits

Sampling procedures were according to the standards described in the German Animal Welfare Act (§4 TierSchG). The implementation of the stow-net fishing for scientific purposes is approved in accordance with § 10 Regulation for the implementation of the Hamburg Fisheries Act in the Elbe estuary by the Authority for the Environment Climate, Energy and Agarwirtschaft (A132- Supreme Fisheries Authority, Stadthausbrücke 12, 20355 Hamburg), by the State Fisheries Office Bremerhaven (Fischkai 31, 27572 Bremerhaven) according to § 10 of the Lower Saxony Coastal Fisheries Ordinance and by the State Office for Agriculture, Environment and Rural Areas of Schleswig- Holstein (Department 3, Fisheries, Hamburger Chaussee 25, 24200 Flintbek). Exemptions to the ordinances on nature reserves Mühlenberger Loch/Neßsand (Amt für Naturschutz, Grünplanung und Bodenschutz, Abteilung Naturschutz, Neuenfelder Strasse 19, 21109 Hamburg) as well as a nature conservation permit to conduct research fishing in protected areas in the NSG "Rhinplate und Elbufer südlich Glückstadt"/FHH area DE 2393-393 "Schleswig-Holsteinisches Elbästuar mit angrenzenden Flächen" from the Office of Environmental Protection (Department of Nature Conservation, Langer Peter 27a, 25506 Itzehoe).

10. Author contributions

RK: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Validation, Writing-original draft, Visualization, Software, Project administration. JT: Investigation, Writing - review & editing. EH: Investigation, Visualization, Writing - review & editing. JW: Supervision, Validation, Writing - review & editing. AF: Funding acquisition, Conceptualization, Supervision, Validation, Writing - review & editing, Project administration. RT, CM: Conceptualization, Funding acquisition, Resources, Writing - review & editing.

Chapter 6 General Discussion

6.1 Spatial and temporal variability of estuarine fish feeding

Spatial variability

The food composition of fishes in estuaries varies primarily due to salinity gradients, which influence both the composition of available prey and the diversity of feeding and foraging habitats. These environmental differences lead to distinct feeding opportunities and dietary patterns among fish species in different parts of the estuary (Koehler et al. 2022, Theilen et al. 2025b).

In the middle estuary of the Elbe (Schwarztonnensand), zander consumed fewer fish and more invertebrates (amphipods, mysids, decapods) than at other stations, likely due to lower availability of smelt, their preferred prey (Theilen et al., 2025b). The preference for smelt as prey aligns with findings by Kafemann and Thiel (1998). Species density data showed that smelt densities were much lower in the middle estuary (Schwarztonnensand: 6,905 per 1 Mio m³) compared to the upper (Mühlenberger Loch: 32,307) and lower regions (Medemgrund: 30,307) (Theilen et al., 2025a). Juvenile zander are flexible predators that prefer fish but switch to less nutritious invertebrates when preferred prey is scarce. This may affect their growth and survival (van Densen, 1985; Vinni et al., 2009; Theilen et al. 2025a, Theilen et al. 2025b). Scarcity of preferred pray may have forced them to consume predominantly less nutritious taxa like mysids (*Neomysis integer*) and amphipods (*Gammarus spp.*) in the Elbe estuary likely affecting their growth.

The diet of smelt differed between the Elbe and Odra estuaries, mainly in the proportions of fish, mysids and gammarids. Smelt in the Elbe consumed fewer fish (IRI: 16.26 % vs. 23.07 %), fewer mysids (19.4 % vs. 53.03 %) and more gammarids (35.61 % vs. 2.07 %) compared to the Odra. Along the Elbe estuary, the diet of smelt shifted along the river course: copepods increased upstream, while mysids declined (Theilen et al. 2025b). *Eurytemora affinis*, the dominant copepod in the Elbe, is mostly concentrated in the freshwater zones near Mühlenberger Loch (Köpcke, 2022). Temperature has a large effect on the reproductive parameters of *Eurytemora affinis*, as population densities were described to decrease drastically when water temperature exceeds 20° C (Devreker et al., 2007; Devreker et al., 2009). Predation on zooplankton can substantially influence their population sizes. In 1992, a collapse of the *Eurytemora affinis* population was observed in the Mühlenberger Loch region, which was likely caused by predation from juvenile fish (Thiel, 2011). According to Mehner and Thiel (1999), smaller fish larvae (4–20 mm in length) exert lower predation pressure on zooplankton such as rotifers, copepodid stages and small cladocerans. However, juvenile fish that exceed 20 mm, have the potential to significantly impact populations of larger copepods and cladocerans. Their enhanced predation success is attributed to their larger mouth gape and more developed abilities to detect and capture prey (Mehner and Thiel, 1999; Thiel 2011). Köpcke (2002) concluded that in the Mühlenberger Loch and the other side-channel areas of the Elbe estuary, populations of *Eurytemora affinis* are regulated through top-down control by predation from fish and *Neomysis integer* starting in May and June.

In the maximum turbidity zone (MTZ), low primary production and poor-quality POM limit food for *E. affinis* (Biederbick et al., 2024; Hauten, 2024). High turbidity favors mixotrophic flagellates, lengthening trophic chain and leading to enrichment of $\delta^{15}N$ (Hauten, 2024; Martens et al., 2024). Enriched $\delta^{15}N$ was found in smelt juveniles in the MTZ (but not in adults), which suggests that adult smelt avoid areas with inappropriate environmental conditions and limited food availability (Hauten, 2024). In Eru Bay (Gulf of Finland), smelt primarily fed on amphipods and mysids, with fish becoming an important prey taxon for adults between August and October (Taal et al., 2014). In the Gulf of Riga, juvenile smelt showed a strong preference for the cladoceran *Bosmina longispina*, but were found to switch to consume alternative prey when preferred food sources were limited (Lankov et al., 2010).

The diet of ruffe shifted along the salinity gradient, with fish becoming more important at lower salinities, particularly in upstream regions where the highest IRI was observed (Theilen et al., 2025b). In the Elbe estuary insect larvae were only found in the diet of ruffe from the middle and upper Elbe estuary. In the Odra estuary, the importance of insect larvae was highest in the upper region and decreased downstream with rising salinity (Theilen et al., 2025b). In the lower Odra region (Pomeranian Bay), insect larvae played a minor role, while annelids, particularly *Hediste diversicolor*, were the dominant prey, declining in importance upstream due to salinity preferences (Bagarrão et al., 2014, Theilen et al., 2025b). In non-tidal estuaries like the Couronian Lagoon, adult ruffe feed on juvenile smelt and perch, along with meso- and macrozooplankton, while in the Darß-Zingst Bodden, they also consume nine-spined sticklebacks (*Pungitius pungitius*) (Hölker and Thiel, 1998). In other freshwater habitats, such as Lake Võrtsjärv and Lake Aydat, chironomid larvae and pupae were the major prey of ruffe (Jamet and Lair, 1991; Kangur and Kangur, 1996). This corresponds with the findings from the Odra estuary (Theilen et al., 2025b), where chironomid larvae were the dominant prey of ruffe in the freshwater area (Odra Lagoon).

In the Elbe estuary, copepods were a more important food source for flounder in the middle and upper regions, likely reflecting the relatively high abundance of *Eurytemora affinis* in the freshwater zone of the southern marginal area (Köpcke, 2002). As migratory fish (Bos, 2000) flounder pass through these areas where copepods are more available. In contrast, flounder in the Odra estuary primarily consumed bivalves (*Mya arenaria*) in the lower region (PB), which includes the sandy Odra Bank, where the benthic community is dominated by this species (Zettler and Gosselck, 2006). Benthic prey made up approx. 50 % of the flounder diet in the Elbe estuary (Drenkelfort, 1994), consistent with observations from other European estuaries such as the Schlei (Nellen, 1968), Westerschelde (Maes, 2000), Humber estuary (Marshall, 1995), Severn estuary (Moore and Moore, 1976), and Tagus estuary (Costa and Bruxelas, 1989). Flounder feeding on copepods and benthic organisms in the Elbe estuary aligns with the findings from other European estuaries (Theilen et al., 2025b). In the Odra estuary, no copepods were found in flounder diets and benthic taxa accounted for a high IRI (84.72 %), indicating an even stronger reliance on benthic prey (Theilen et al., 2025b). Seasonal dietary shifts have been observed in flounder from the Åland archipelago (northern Baltic Sea) (Aarnio et al., 1996), while in the southwestern Baltic, *Limecola balthica* is the main prey species (Haase et al., 2020).

Differences in food composition between the two estuaries reflect both prey availability and the generalistic feeding behaviour of the keystone species, which exploit a range of food resources. As a result, local fluctuations in prey abundances lead to distinct dietary patterns. The Elbe and Odra estuaries differ in their ecological and hydrological characteristics: the Elbe is a mesotidal system with strong tidal currents and high flow velocities, driving daily salinity fluctuations. In contrast, the Odra is a microtidal estuary with a lagoon-like structure and longer water residence times in the Odra Lagoon (Pein and Staneva, 2024), supporting different prey compositions. In the Elbe estuary copepods dominated in the southern marginal areas in the freshwater zone (Köpcke, 2002), whereas the shallow areas close to the mouth of the river Odra were dominated by *Mya arenaria* (Darr et al., 2014), while insects reproduce in large numbers under lower salinities (Williams and Williams, 1998) of the Odra Lagoon.

Temporal variability

Most studies capture only a brief snapshot of food availability, typically during summer when stream flows are low and temperatures are high, thereby neglecting important seasonal variations that can significantly affect fish growth (Armstrong et al., 2021; Bacon et al., 2004; Ouellet et al., 2024). In this regard, samples in this study were collected during all four seasons. Seasonal variations in salinity, temperature and freshwater inflow have influences on the reproductive cycles of both predators and prey in estuarine ecosystems. Temperature affects the metabolic rates of fish, thereby altering their feeding demands (Volkoff and Rønnestad, 2020). Seasonal fluctuations of the river discharge, modify the salinity gradient dynamically and affect the distribution of aquatic organisms. These discharge changes particularly influence plankton dynamics, as higher flow rates increase the downstream transport of planktonic species (Steidle and Vennell, 2024). Generalist predators respond opportunistically to these environmental shifts, adapting their diets based on the seasonal availability of prey (Costalago et al., 2012). The combination of seasonal shifts in temperature, salinity, and discharge also affects spawning and recruitment processes of fish, thereby influencing fish community structure (Thiel et al., 1995; Thiel, 2011; Kellnreitner et al., 2012; Eick and Thiel, 2014). In the Elbe estuary, for instance, smelt spawning migrations in late winter are triggered by increased river discharge (Lillelund, 1961).

Seasonal changes in the diet of zander were more pronounced in the Odra estuary. While food composition in the Elbe remained relatively similar between summer and autumn, zander in the Odra estuary consumed fewer fish and a broader range of prey in autumn, including benthic invertebrates such as annelids, gastropods and isopods. The proportion of decapods also increased, suggesting a reduced availability of preferred fish prey in autumn (Theilen et al., 2025b). Studies from the Kiel Canal (NOK) by Kafemann and Thiel (1998) reported reduced growth of zander due to a lack of sufficient fish prey, particularly when prey such as herring (Winkler, 1980) and smelt (van Densen, 1985; Buijse and Houthuijzen, 1992) is missing.

In the Elbe estuary, seasonal shifts in smelt diet were most evident in winter, when copepods (especially *Eurytemora affinis*) were the dominant prey, indicating higher copepod availability in late winter. Compared to findings from the years 1991–1993 (Thiel, 2001), smelt in 2021–2022 (Theilen et al., 2025b) showed less cannibalism (IRI = 11.79% vs. 23.74%) and consumed fewer fish overall (IRI = 16.27% vs. 26.03%) in the Elbe estuary. Lüchtenberg (1986) described cannibalism in smelt from the Elbe estuary, noting that cannibalism rates increase over the year, being low in early spring (March, 0.9 %) and reaching higher levels by August (11.4 %). Gammarids were more important in the recent period (IRI = 35.61% vs. 3.08%), while mysid proportions remained similar. The shift towards an increased consumption of amphipods, serves as indicators for an altered food web in the Elbe estuary. The proportion of *Eurytemora affinis* in the diet of smelt generally declined (IRI = 4.77% in 2021–2022 vs. 11.78% in 1991–1993), suggesting reduced copepod availability (Theilen et al., 2025b). Historically described as plankton feeders dependent on *Eurytemora affinis*, smelt were also reported to shift to benthic prey such as gammarids during winter starvation periods (Ladiges, 1935). In 2021–2022, however, gammarids were a major food source year-round, pointing to a long-term decline in preferred prey. The low primary production and poor POM quality in the Elbe’s maximum turbidity zone (MTZ) likely constrain zooplankton resources. Stable isotope data (Hauten, 2024) further suggest that juvenile smelt remain in the MTZ despite poor conditions, while adults avoid it. Frequent ingestion of indigestible items like sand and plant debris, especially in winter, supports the idea of seasonal food limitation. Smelt in the Odra estuary fed primarily on fish during late summer, particularly gobies of the genus *Pomatoschistus*. As the availability of gobies declined by October, mysids became the main prey (Theilen et al. 2025b).

Seasonal diet variation in ruffe was less pronounced compared to the other keystone species from both estuaries. Amphipods were consistently consumed across all seasons, although mysids were absent in the diet of ruffe caught during winter season in both estuaries (Theilen et al., 2025b). In the Odra estuary, ruffe fed primarily on annelids in autumn and on cladocerans in summer, likely reflecting seasonal peaks in cladoceran abundance due to temperature and salinity conditions in the Baltic Sea (Möllmann et al., 2002; Theilen et al., 2025b). A comparison with earlier studies of ruffe from the Elbe estuary revealed changes in the food composition: In 1991–1993, ruffe consumed a significant proportion of *Eurytemora affinis* copepods (IRI = 26.21 %) (Thiel, 2001), which were entirely absent in 2021–2022 specimens (Theilen et al., 2025b). The importance of gammarids increased substantially (IRI = 48.43 % vs. 7.5 5%). Unlike in the 1990s, fish (mainly smelt) were present in the diet of ruffe (IRI = 6.5 %), indicating a shift in prey availability or food selection (Theilen et al., 2025b).

The diet composition of flounder showed the largest variability among the keystone fish species, reflecting its generalist feeding behaviour (Teichert et al., 2022). In the Elbe estuary, copepods were a major dietary component during spring and winter, while in the Odra estuary, annelids dominated in spring and winter, and bivalves were more dominant prey in summer and autumn. This seasonal shift likely reflects a preference for annelids, which are easier to digest due to their lack of a hard shell. Although the prey composition of flounder varied between the 1991–1993 (Thiel, 2001) and 2021–2022 periods (Theilen et al., 2025b), the differences were less pronounced compared to other species. In both periods, copepods were a major part of the diet in the Elbe estuary (IRI 1991–1993: 41.29 %, IRI 2021–2022: 34.36 %), while zoobenthos species increased from 14.4 % (1991–1993) to 24.7 % (2021–2022). Together, copepods and other zoobenthic taxa made up more than 50 % of flounder's diet in both periods in the Elbe estuary (Theilen et al. 2025b).

6.2 Competition for food resources

When food resources are scarce, they are often not shared equally among competing organisms (Rajaniemi and Reynolds, 2004; Thompson and Fox, 1993; George and Collins, 2024). Species that are larger, more aggressive or faster growing, emerge earlier or reproduce fast tend to gain disproportionate access to food or habitat. This can lead to asymmetric competition, where some individuals or species experience significantly greater negative effects than others (George and Collins, 2024). Within a species, competition for food can be a strong evolutionary force, favouring individuals that forage more efficiently over their less successful counterparts (Ward et al., 2006).

To further quantify differences and similarities in diet composition among estuarine fish species, hierarchical clustering showed two main groups at a dissimilarity of approx. 60 % (Figure 6.1). The first cluster was formed by zander (E, O), smelt (E, O) and ruffe (E), since those species consumed fish, amphipods and mysids. The first cluster was further divided into smelt and ruffe from the Elbe estuary and flounder from the Elbe estuary. The smelt/ruffe (E) subcluster was formed due to the dominance of amphipods in both diets and the similar proportions of fish, mysids and decapods. The first cluster was further divided by a separation of zander (E, O), since zander from both estuaries consumed approx. 50 % fish. The diets of flounder and ruffe from the Odra estuary were separated from the first cluster. The flounder/ruffe subcluster was formed due to their shared consumption of insects, decapods, mysids, amphipods and annelids. These cluster patterns suggest that trophic similarities are influenced not only by species identity but also by estuarine-specific prey availability and habitat characteristics. The distinct grouping of flounder and ruffe from the Odra estuary likely reflects localized prey communities and feeding opportunities. the consistent clustering of zander from both estuaries, driven by their high fish consumption, underscores their role as top predators in both estuaries. The clustering of smelt and ruffe from the Elbe estuary can be attributed to their similar consumption of amphipods, mysids, and decapods, indicating potential competition for shared food resources (Theilen et al. 2025b).

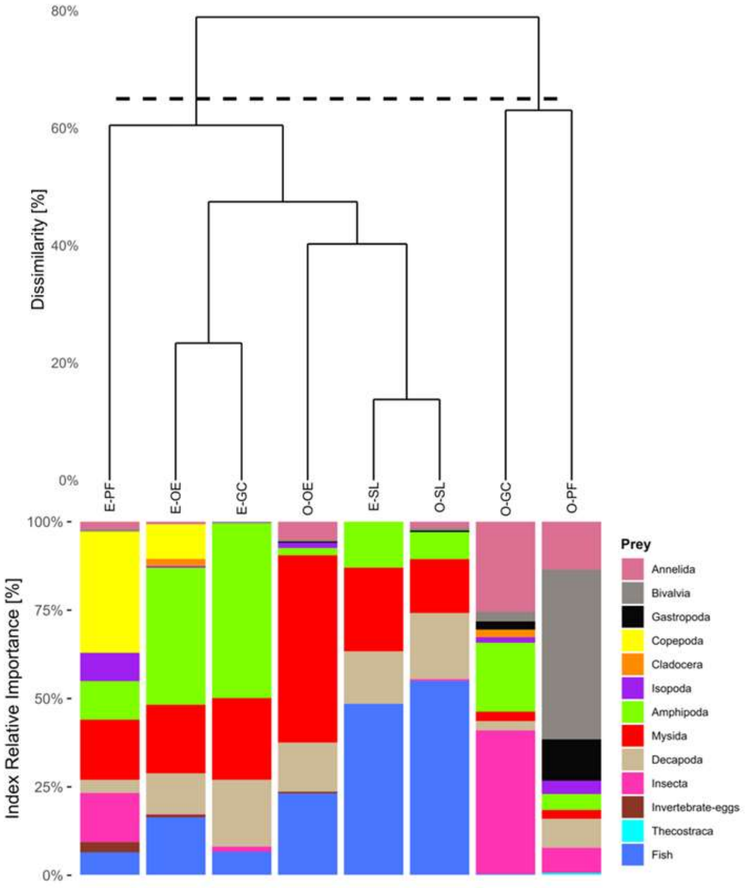

Figure 6.1: Hierarchical clustering of diet composition from keystone fish species (SL = *Sander lucioperca*, OE = *Osmerus eperlanus*, GC = *Gymnocephalus cernua*, PF = *Platichthys flesus*) from the Elbe (E) and Odra (O) estuaries on Bray-Curtis dissimilarity. General diet composition described by the index of relative importance (IRI %) of each individual keystone species and the respective estuary. Dashed line at 70 % dissimilarity.

Sharing an abundant food source does not necessarily indicate direct competition; it may instead reflect high availability of certain prey items that drives food selection (Stanford et al., 1986). Schoener's index values above 0.6 or below 0.4 are typically considered ecologically significant (Matthews and Hill, 1980; Wallace, 1981; Galat and Vucinich, 1983; Muth and Snyder, 1995; Minder, 2023). The comparison of dietary overlap among keystone fish species in the Elbe estuary between 2021–2022 (Theilen et al., 2025b) and historical data from the 1990s (Thiel, 2001) showed generally lower overlaps in the 1990s, largely due to the increased presence of amphipods in the diet from recent samples. The observed changes in the the two species zander, smelt and ruffe, which are widely distributed along the entire Elbe estuary, suggest that the food availability for these fish species has shifted between the 1990s and 2021/2022 (Kafemann & Thiel, 1998; Thiel, 2001; Theilen et al., 2025b). Although it cannot currently be definitively concluded that the reduced food availability in the Elbe estuary is solely responsible for the presently lower growth of the ruffe, the change in food availability is very likely a contributing factor (Chapter 3). The high dietary overlap (0.703) between smelt and ruffe in the Elbe estuary suggests ecologically relevant competition. These findings point either to competition for amphipods due to limited availability of alternative prey or to an overall high abundance of amphipods during the study period. Limited availability of preferred prey would indicate a degradation of the food web in Elbe estuary.

6.3 Nutritional requirements for fish diets

Due to their distinct life histories and prey preferences, different fish species can have varying nutritional requirements. The growth of fishes is dependent on the sustained supply of food resources. Periods with generally lower food availability or absence of nutritious prey organisms can directly lead to decelerated growth in fish (Amundsen et al., 2007). According to Ouellet et al. (2024), food quality may be equally important to food quantity in riverine ecosystems. The food resources available to fish in rivers differ in energy and nutrient content. There is a wide variation in the biomass, digestibility, and energy density across organisms from different taxonomic groups (Cummins and Wycheck, 1971; Ouellet et al., 2024).

The diet of fish includes macronutrients such as proteins, lipids, and carbohydrates, as well as micronutrients like minerals (e.g. calcium, magnesium, iron) and vitamins. According to Wilson (2003), the major organic compound of fish tissue is protein, making up to 65 % to 75 % of the total dry weight. Consumed protein is used to obtain amino acids which are used to synthesise new protein (for growth and reproduction) or to replace existing protein (maintenance), therefore a consistent intake of protein is necessary. Inadequate protein in the diet results in growth reduction since protein from less vital existing tissues is used to maintain vital functions. In fish, the liver plays a key role in regulating and mobilizing energy reserves, primarily using amino acids as a fuel source (French et al., 1981, Falco et al., 2020, Jia et al., 2021).

The amount of dietary protein is crucial for fish growth, as protein is the primary macronutrient supporting tissue development (Wilson, 2003). Studies have confirmed that optimal protein requirements vary among fish species and that inadequate or imbalanced protein sources can restrict weight gain and reduce growth efficiency (e.g. Park et al., 2022; Wang et al., 2023; Wiechetek et al., 2025).

The feeding analysis in this thesis showed that amphipods were an important part of the diet, especially in smelt, ruffe and zander from the Elbe estuary. When comparing the food composition of these three species with data from the 1990s, it is evident that amphipods have become a more prominent component of their diet, potentially leading to an imbalance in optimal protein content and consequently, reduced survival and growth. For zander, this pattern is noticeable on a spatial scale within the Elbe estuary: in the maximum turbidity zone of the middle estuary where amphipod consumption was highest, growth and condition factor were lowest (Koll et al., 2024; Theilen et al., 2025b).

Abo-Taleb et al. (2021) substituted fish meal by amphipod meal extracted from *Gammarus pulex* and found that weight gain of reared fish (*Mugil cephalus*) was reduced when fish meal was replaced by amphipod meal in a formulated diet, indicating that amphipod meal was inferior to fish meal. The biochemical analyses by Abo-Taleb et al. (2021) showed that dry weight of fish meal (60.17 %) had higher crude protein level compared to amphipod meal (40.02 %), but less amounts of nitrogen free extracts (fish meal: 11.5 %, amphipod meal: 24.51 %), and ash (fish meal: 15.4 %, amphipod meal: 19.82 %). Amphipods have a sturdy cuticula, which is formed by chitin and calcium (ash i.e. amount of inorganic non-combustible material), which makes them of lower nutritional value compared to other prey organisms with no cuticula such as fish or less sturdy cuticula such as mysids or copepods. Biochemical analyses by Raymont et al. (1968) showed that protein content of *Neomysis integer* was 71 % and ash (including chitin) 14 % of the dry weight.

While the importance of amphipods in the diets of smelt, ruffe, and zander has increased compared to the 1990s in the Elbe estuary, the proportions of calanoid copepods (*Eurytemora affinis*) and mysids (*Neomysis integer*) have declined. This dietary shift may result in an overall lower intake PUFAs, leading to inadequate nutrition that could have negatively affected growth and reproduction success of these species. All vertebrate species have dietary requirements for certain poly unsaturated fatty acids (PUFA), which belong to the so-called essential fatty acids (EFA), including linoleic acid, 18:2n-6 (omega-6 fatty acids) and alpha-linolenic acid, 18:3n-3 (omega-3 fatty acids). PUFA are almost exclusively synthesized by plants, which are transferred to highly trophic levels in a planktonic food web and are accumulated in the higher trophic levels (Brett and Müller-Navarra, 1997; Bandara et al., 2023). High contents of PUFA, which includes all varieties of highly unsaturated fatty acids, are especially found in fish and shellfish (Ackman, 2007; Brett and Müller-Navarra, 1997). Therefore, preying on other fish or other organisms from the planktonic food web comes with nutritional advantages. Highly unsaturated fatty acids (HUFA) are a specific group of PUFA, which have 20 or more carbon atoms and 3 or more double bonds (Olsen, 2009). Long-chain PUFAs are mainly produced by certain algae, such as diatoms and cryptophytes and transferred through the food web to fish (Taipale et al., 2013). Stream diatoms are rich in long-chain PUFAs eicosapentaenoic acid (EPA, 20:5n-3) but low in docosahexaenoic acid (DHA, 22:6n-3) and differ from marine and lake species (Torres-Ruiz et al., 2007; Brett et al., 2009). Green algae offer moderate nutritional quality due to alpha-linolenic acid (ALA, 18:3n-3) and linoleic acid (LIN, 18:2n-6) content, while cyanobacteria lack long chain PUFAs, making them poor food for invertebrates (Martin-Creuzburg et al., 2008; Lang et al. 2011). As herbivorous invertebrates cannot synthesize long-chain PUFAs efficiently, their PUFA profiles reflect those of their algal diet, ultimately affecting the nutritional quality for secondary consumers, such as fish (Brett and Müller-Navarra, 1997; Guo et al., 2017). Evjemo et al. (2003) found that especially the calanoid copepods *Temora longicornis* and *Eurytemora affinis* have higher total HUFA content, extending to 55-62 % of the total fatty acids. Diet of *Neomysis integer* in the Elbe estuary was dominated by calanoid copepod *Eurytemora affinis*, while being supplemented by rotifers, cladocerans, phytoplankton and benthic organisms (Fockedey and Mees, 1999). Higher concentrations of suspended particulate matter (SPM) have been shown to impact lower trophic levels, primarily by limiting phytoplankton production due to reduced light availability (de Jonge et al., 2014; Steidle and Vennell, 2024). Findings from Bernát et al. (1994) demonstrated that higher SPM levels impaired the filtration efficiency of the calanoid copepod *Eurytemora affinis* during both naupliar and adult stages. The recent increase in SPM concentrations in the freshwater regions of the Elbe estuary may have affected copepod densities, potentially disrupting the entire food web. Similar effects were described by Shoup and Wahl (2009), in which the decreased densities of zooplankton and benthic invertebrates ultimately restricted food availability for fish. Many secondary consumers will have optimum growth, when there is a sufficient amount of long chain PUFA is directly available in their diet. High retention of long chain PUFA in the secondary consumers results in higher availability in the next trophic level (Brett and Müller-Navarra, 1997).

Even non-specialist predators sometimes consume prey organisms that are hard to digest, like hard-shelled invertebrates, especially when higher quality food is scarce or inaccessible. According to the optimal foraging theory (Stephens and Krebs, 1986), costly prey can become favourable when the net energy gain outweighs that of other available feeding options, especially under conditions like resource depletion, competition or increased predation risk (Langerhans et al., 2021).

6.4 Effects on fish growth

Growth is determined by a range of factors, including environmental conditions, genetic characteristics, dietary composition and health. Fishes grow continuously throughout their lives, although growth rates tend to slow down as they reach maturity (Ali et al., 2003; Kozlowski, 1996; Ziegelbecker and Sefc, 2021). They are ectotherms and therefore their metabolic rate is strongly affected by the water temperature (Volkoff and Rønnestad, 2020). Warmer temperatures generally increase the metabolic activity leading to faster growth, and colder temperatures slow down the growth. Excessively high temperatures can cause stress, leading to health issues that ultimately inhibit growth (Volkoff and Rønnestad, 2020). Fish express their optimum growth within a specific temperature range. However, thermal stress (particularly at temperatures above the species' optimum) can lead to physiological disturbances, reduced growth, and a higher risk of disease outbreaks (Brett, 1971; Farrell, 2002; Alfonso et al., 2020).

Liver transcriptomes indicated that zander from highly turbid areas were experiencing starvation, which was supported by their compromised body condition and the respective growth. Gene expression patterns (activation of autophagy and fat degradation pathways in the liver) in zander from the maximum turbidity zone (middle estuary) matched with lower Fulton's condition factor (K) compared to those at other stations and a relative increase in invertebrate (amphipods, mysids, decapods) consumption compared to fish. This indicated that environmental conditions and food availability were unfavourable for juvenile zander in the middle estuary (Koll et al., 2024; Theilen et al., 2025a). As densities of smelt, which is the preferred prey of zander (Kafemann and Thiel, 1998, Theilen et al. 2025b), were substantially lower in the middle region compared to the upper region and the lower region (Theilen et al., 2025a). These observations match with findings by van Densen (1985), who reported substantial growth differences between piscivorous and non-piscivorous juvenile zander. In the middle region instead of fish, the juvenile zander consumed more invertebrates (amphipods, mysids, decapods), which has led to stunted growth in that area.

It is widely recognized that healthier fish tend to grow faster, as they require less energy for maintenance and immune responses, allowing more energy to be invested in growth (Esmaeili, 2021). Sepulveda (1994) found, that a concentration of below 4.5 mg/L dissolved oxygen already causes reduced growth in smelt larvae. In the oxygen minimum zone (OMZ), which is in the freshwater region of the Elbe estuary, dissolved oxygen concentration regularly falls below 5 mg/L during summer (FGG-

Elbe, 2024; Theilen et al., 2025a). This reoccurring hypoxia could have impaired the growth of smelt in recent years. Despite the oxygen depletion in summer, in this area the highest densities of juvenile zander are found (Theilen et al., 2025a). The found cellular stress responses, immune activation, disruption of the core microbiome and elevated levels of potentially pathogenic bacteria, which were observed, were not directly linked to compromised biometric indicators (body condition). However, zander from the maximum turbidity zone showed signs of starvation, reflected by poor body condition and activation of liver autophagy and fat degradation pathways. Similar effects are likely to also impact other fish species that hatch or develop in this region, several of which have already shown declining biomass in the Elbe estuary (Scholle and Schuchardt, 2020; Koll et al. 2024; Theilen et al., 2025a). As oxygen deficiency events are expected to increase with climate change, this study highlights the potential rise in disease risks, including shewanellosis (Koll et al., 2024). The interaction between host and the microbiome is gaining growing attention in the evaluation of individual fish health (Kelly and Salinas, 2017). In recent studies the bacterial composition of wild fish gills was investigated for an assessment of pathogen presence and identifying potentially pathogenic species (Pratte et al., 2018; Itay et al., 2022; Minich et al., 2020). In our study (Koll et al., 2024), *Shewanella* strains, including *S. putrefaciens* and *S. baltica*, showed the strongest correlations with immune responses in fish samples, while remaining rare in the water column. *Shewanella* has the ability to degrade organic matter under hypoxic condition (Nealson and Scott, 2006). Repeated detection in diseased fish, combined with *S. putrefaciens* infection indicates the high pathogenicity (Cocchi et al., 2018; Paździor et al., 2023; Rusev et al., 2016). Infection rates were shown to be highest in European eel (*Anguilla anguilla*) when dissolved oxygen concentrations dropped below 5 mg/L (Esteve et al., 2017).

Fish growth can serve as an indicator of food availability, as increased food intake generally leads to faster growth (Goodrich and Clark, 2023). An undernourished fish lacks the nutrients necessary for proper growth, resulting in impaired development of bones, muscles and internal organs. Naturally, fish undergo periods of reduced food intake as part of their life cycle, during which energy reserves are redirected from growth toward maintaining essential physiological functions (Sumpter et al., 1991). Hypoxia has been shown to affect feeding behaviour in several fish species (Pichavant et al., 2001; Eby et al., 2005; Chabot and Claireaux, 2008). The hypoxic conditions primarily reduce foraging intensities and metabolic rates, which leads to slowed growth (Chabot and Claireaux, 2008). There have been several authors that have documented the oxygen situation in the Elbe estuary over the last 40 years (Riedel-Lorjé and Gaumert, 1982; Möller, 1988; Möller and Scholz, 1991; Thiel et al., 1995; Thiel, 2011; Eick and Thiel, 2014). Within this time frame, severe hypoxic conditions occurred especially in the Hamburg Port region (freshwater areas). Compared to the 1980s, the oxygen concentration improved, however, in recent years the oxygen conditions have deteriorated again, which led to reoccurring hypoxic events during summer months (FGG-Elbe, 2024). These hypoxic events could have impaired the foraging behaviour of the keystone fish species, as hypoxia can reduce feed intake, growth, and oxygen consumption in fish (Pichavant et al., 2001; Magnoni et al., 2018). Changes in feeding strategies can also occur under different turbidity conditions, with visual predators being more affected by increased turbidity than fish that feed on benthic macroinvertebrates (Hecht and van der Lingen, 1992). For visual predators, the turbidity of the water column plays a crucial role in their predation success. Increased turbidity could also explain the dietary shift towards extended feeding on benthic invertebrates (amphipods). Generally, zander and smelt feed on more pelagic organisms, particularly smelt, who primarily consume calanoid copepods, and zander, who primarily feed on other fish. However, as turbidity increased (FGG-Elbe, 2024), visual predation might have been impaired, causing a shift towards more benthic foraging (Theilen et al. 2025b). Notably, during 2021–2022, zander consumed gammarids at nearly all stations (except MG at the river mouth), whereas gammarids were completely absent from their diet in the Elbe estuary in 1992–1993 (Kafemann and Thiel, 1998; Theilen et al., 2025b), which led to inferior growth. It is suspected that these shifts in food composition were induced by the availability of prey, with changing foraging behaviour as a result of altered environmental conditions. The comparison of recent and historical diet data for keystone fish species reveals a shift in the Elbe estuary from a predominantly plankton-based food web to one that is more reliant on benthic organisms. This may reflect ecological responses to long-term environmental changes and anthropogenic alterations in the estuary.

In addition to the abundance of accessible prey species, their nutritional quality plays a crucial role in food assimilation and the respective growth (Ouellet et al., 2024). Assimilation efficiency is the proportion of ingested food that an organism is able to digest and absorb into its body for use in metabolism, growth and reproduction. According to Welch (1968), net growth efficiency is negatively correlated with assimilation efficiency and may range from 20 % to 90 %, while gross growth efficiencies typically lie between 15 % and 35 %. Carnivorous species generally exhibit high assimilation efficiency but lower net growth efficiency, whereas herbivores and detritivores have lower assimilation efficiencies and higher net growth efficiencies (Welch, 1968). The diet composition also influences assimilation efficiency in fish, with lipid-rich prey enhancing energy uptake, while prey with high cellulose content (e. g. algal cell walls) reduces assimilation efficiency (Teferra, 2003).

Costly prey consumption can be caused by animals optimizing under poor conditions (optimal foraging). Once high-quality food becomes available, the growth enters a compensatory growth phase. Durophagy, the consumption of hard-shelled prey, can impose costs such as reduced somatic or reproductive growth due to a high proportion of indigestible material and limited gut space, as hard-shelled prey is digested slower (Langerhans et al., 2021). This corresponds with our findings, as we observed a shift in the diet within the Elbe estuary from easily digestible prey such as mysids and copepods (1990s) to harder-shelled prey like amphipods (2020s), which could have affected the growth of the keystone species.

6.5 Fish fauna composition in a mesotidal estuary

Smelt and twaite shad are the most abundant species in the anadromous life cycle guild (LCG) in the Elbe estuary (Theilen et al., 2025a). Both species have faced a reduction of their stock size in the Elbe estuary in recent years. Between 2009-2010 and 2021-2022 mean densities of the anadromous LCG dropped by over 92%, from 20,000 to 1,500 individuals per million m³. The decline of the anadromous LCG, was mainly due to smelt but also species such as twaite shad and three-spined stickleback contributed to this finding (Theilen et al., 2025a). In the Elbe estuary smelt alone comprising up to 96 % of the fish fauna (Eick and Thiel, 2014). Smelt begin aggregating at the river mouth between November and February in preparation for their spawning migration, which occurs from February to March (Lillelund, 1961; Borchardt 1988; Kottelat and Freyhof, 2007). This temperature-driven migration (Kottelat & Freyhof 2007) ensures that larval development is well synchronized with peak local food availability (Sepulveda et al. 1993, Köpcke 2002). The decline of several fish species likely results from multiple stressors including increased oxygen deficiency, higher suspended particulate matter, starvation and reduced shallow nursery habitats (Scholle and Schuchard, 2020; Illing et al., 2024; Koll et al., 2024; Theilen et al., 2025a). Simultaneously, lower river runoff (32 % below the long-term average between 2014 and 2020) and an enlargement of the channel have intensified marine water intrusion, favouring inland migration of marine species (Zhu et al., 2014; Ralston and Geyer, 2019; Rewrie et al., 2023). Consequently, the fish guild structure in the Elbe estuary experiences a shift to that of macrotidal UK estuaries, where diadromous species are less important (Elliott and Dewailly, 1995; Theilen et al. 2025a).

Over the study period, environmental factors strongly shaped fish community structure in the Elbe estuary. Electrical conductivity, reflecting salinity, had the greatest influence with higher salinity zones near the river mouth supporting more marine species consistent with earlier findings of Thiel and Potter (2001) and Eick and Thiel (2014). Ongoing sea level rise and declining river runoff are expected to intensify marine influence (Little et al., 2022; Pein et al., 2023). Seasonal changes in species composition were linked to temperature, migration and spawning (Theilen et al., 2025a). The high densities of diadromous species like smelt, twaite shad, and three-spined stickleback lead to seasonal shifts in species composition during migration and nursery periods. Smelt densities peaked from early June to late August, dominated by young-of-the-year individuals. Three-spined stickleback were more abundant in colder months, while common bream (freshwater species) densities in the upper sections increased during warmer summer temperatures. Anadromous migration of smelt and twaite shad was associated with rising temperatures and higher runoff (Lillelund, 1961; Aprahamian et al., 2003). The densities of freshwater species (ruffe) showed a drastic decline (Theilen et al. 2025a). In 2009, Freyhof et al. (2023) considered ruffe a beneficiary of bank stabilization measures, predicting a long-term population increase with temporary stability. However, this assessment is no longer supported. More recent evaluations indicate a moderately declining short-term population trend, and ruffe is now included on the early warning list (Freyhof et al., 2023). In the 1980s, poor water quality, caused by high nutrient loads and frequent hypoxic events, negatively impacted fish. Severe oxygen deficits (even anoxia) led to local complete absence of smelt (Möller and Scholz, 1991). Early oxygen depletion during critical life stages can wipe out entire smelt generations (Möller and Scholz, 1991; Thiel et al., 1995). Oxygen conditions generally improved after 1990, particularly in the lower and middle estuary at Brunsbüttel, Schwarztonnensand and Twielenfleth, but critical dissolved oxygen levels persisted in the upper areas at Mühlenberger Loch during summer, continuing to effect larval growth (Sepulveda, 1994).

Historically, nutrient loading and eutrophication led to oxygen depletion and collapse of entire fish cohorts in the Elbe estuary (Möller and Scholz, 1991). While fish stocks recovered after water quality improvements in the 1990s, recent surveys showed declines in several key species since 2009–2010. Intensified dredging has amplified tidal pumping, increasing suspended particulate matter (SPM), which harms eggs and larvae (Yang et al., 2019) and alters smelt gill tissues, may increase mortality and impair growth (Thiel and Pezenburg, 2007). SPM peaks have shifted upstream, indicating a transition toward macrotidal dynamics (de Jonge et al., 2014). Especially affected are side channels and shallow nursery habitats like Mühlenberger Loch, formerly high in copepod prey density (Thiel, 2001; Köpcke 2002). Through tidal pumping organic-rich sediments are transported upstream. These particles include organic carbon and active microorganisms, that break down the organic compounds and increase oxygen demand, which contributes to summer oxygen deficiencies (Tobias-Hunefeldt et al., 2024). Rising SPM concentrations at Schwarztonnensand indicated an upstream migration of the maximum turbidity zone (MTZ). According to Lang (1990), mesotidal estuaries typically have peak SPM in the gradient zone, while macrotidal estuaries exhibit higher SPM in freshwater areas. The observed upstream MTZ migration, coupled with increased tidal amplitude, suggests, that the Elbe estuary is transitioning toward macrotidal conditions. The southern side channels between Twielenfleth and Mühlenberger Loch, including Hahnöfer Nebenelbe, Lühesander Süderelbe and the Mühlenberger Loch, were identified as the estuary's most productive fish habitats (> 200 kg/ha/year; Thiel, 2001), but are now impaired by sediment loads and oxygen deficiencies. Invasive species, such as round goby (*Neogobius melanostomus*) established in the Elbe estuary since 2008, compete with native fish for resources and prey on their eggs, but have also become a year-round food source for predators like zander (Hempel et al., 2016). While several fish species in the Elbe estuary were negatively affected, marine-estuarine opportunists like herring and whiting have benefited from shifting environmental conditions. The increased intrusion of marine waters has expanded their accessible habitat and whiting, a Lusitanian species, has shown increasing densities in the German North Sea (Thiel et al., 2025). Although peaks of juvenile whiting are not annual, rising temperatures due to climate change are likely to make their occurrence in the Elbe river mouth more frequent.

6.6 Conclusions

The differences in the diet of keystone fish species were highlighted, in which the variation in the Elbe and Odra estuaries were probably driven by prey availability. Seasonal changes in food composition was reflected by fluctuations in prey availability, with opportunistic predators adapting their diets accordingly. This was most evident in flounder, who had the highest diet breadth amoung the keystone species. Compared to the 1990s, the Elbe estuary now shows increased amphipod consumption in zander, smelt, and ruffe, suggesting a shift from a plankton-based to a more benthic-oriented food web. Together with trophic elongation of the food chain, these shifts could have been induced by changed environmental factors, which may have ecological impacts on phytoplankton and zooplankton that ultimately affect higher trophic levels, promoting altered foraging behaviour. The increased dietary overlap among species in the Elbe estuary further suggests intensified competition, likely due to limited food resources.

For smelt, ruffe, and flounder, the asymptotic maximum lengths were larger in the Elbe estuary compared to the Odra, while the growth coefficients were smaller in the Elbe. Zander, smelt and ruffe also showed slower growth compared previous studies in the Elbe estuary. Inferior growth was observed particularly for juvenile zander in the Elbe estuary compared to the Odra estuary. The growth of juvenile zander was particularly affected in the maximum turbidity zone in the middle estuary in the Elbe. The slower growth observed in these regions appears to be associated with a reduced intake of fish prey. Here, the juvenile zander consumed more invertebrates such as amphipods, mysids and decapods. These results suggest that altered environmental conditions in the Elbe estuary are influencing fish growth patterns, emphasizing the need for continued monitoring to further validate underlying causes.

The quantitative long-term analysis of environmental impacts on fish community composition in the Elbe highlighted the strong influence of human-altered factors on estuarine fish fauna. Water quality improvements in the 1990s and early 2000s initially boosted fish densities. Recent conditions, which are marked by increased suspended particulate matter, upstream migration of the maximum turbidity zone, reduced runoff and summer hypoxia have severely affected fish stocks in the Elbe estuary. Mean fish densities dropped by over 90% between 2009–2010 and 2021–2022, primarily affecting smelt populations. These findings highlight the critical importance of the protection estuarine fish nurseries to maintain ecosystem services, which are currently undermined by ongoing ecological degradation.

The integration molecular data from gill and liver tissues, key organs for immune function and metabolic stress response, together with the gill mucus microbiota composition, physiological and abiotic data, was used to identify stress on estuarine fish at high resolution. Zander from the maximum turbidity zone showed signs of starvation, reflected by poor body condition and activation of liver autophagy and fat degradation pathways. In contrast, zander from the upper estuary living under higher temperatures, nutrient loads and low oxygen exhibited, both adaptive and immune responses, linked to specific bacterial taxa. Many of the observed stress and immune pathways were previously undocumented for this species. Overall, the study demonstrated the feasibility of using field-collected molecular data for monitoring fish health in estuarine environments.

This thesis highlights fish ecology in estuarine ecosystems from four perspectives: (1) fish population dynamics and species composition, (2) food composition emphasizing the ecological roles of individual keystone species, (3) growth analyses as indicators of food availability and habitat quality and (4) stress responses at the individual level, using zander as representative for the keystone fish species. These findings provide evidence of altered environmental conditions in the Elbe estuary as reflected in shifts in species composition, changes in prey consumption and reduced growth compared to earlier studies. Together, these insights contribute to a better understanding of ecological processes and stressors in estuarine ecosystems.

6.7 References

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