

ORIGINAL RESEARCH ARTICLE

Are fatty acids in fish the evidence of trophic links? A case study from the southern Baltic Vistula Lagoon

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Abstract Most knowledge on the feeding ecology of fish has been based on the analyses of food remains from the alimentary tracks. This traditional method, however, only provides information about recently consumed food, and is burdened with a risk of incorrect assessment of the role of individual diet components due to the different rates of digestion. A method free from such limitations is the analysis of fatty acids. The objective of our study was to recognise the potential of fatty acid signatures in providing information on the diet and feeding habits of six fish species from the shallow brackish Vistula Lagoon, southern Baltic Sea (*Anguilla anguilla*, *Abramis brama*, *Rutilus rutilus*, *Pelecus cultratus*, *Perca fluviatilis*, *Sander lucioperca*). Multivariate statistical analyses of fatty acid signatures permitted relevant grouping of the fish according to species and their diet, as well as evidenced substantial ontogenetic changes in perch, roach, and bream. They might be caused by dietary changes but can also result from internal regulatory processes. The obtained results confirmed that fatty acids provide useful, time-integrated dietary information, contributing to expanding knowledge regarding the feeding ecology of fish in shallow coastal water ecosystems. They also pointed to the necessity of assessment of the invertebrates and fish's ability to perform endogenous synthesis of polyunsaturated fatty acids, particularly in research on benthic communities. To our best knowledge, this is the first attempt to investigate the feeding habits of fish and food-web relationships in the coastal waters of the Baltic Sea using fatty acids.

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1. Introduction

Most knowledge on the feeding ecology of fish has been achieved through dietary studies based on morphological identification of undigested food remains from the alimentary tracks (Amundsen and Sanchez-Hernandez, 2019; Hyslop, 1980). This traditional method provides essential information to understand the biology of fish species and their ecological role in the aquatic system, important in planning and implementing appropriate measures to protect biota

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and ecosystems. Although stomach content analysis prevails as the basic source of information on the diet and feeding habits of fish, it has some considerable limitations. Firstly, such an approach provides information only about recently consumed food items, and single estimates may not give an accurate picture of the diet from a longer perspective. Secondly, stomachs may be empty at the time of capture or via regurgitation (Arrington et al., 2002; Sutton et al., 2004). Moreover, due to differential rates of digestion of different types of prey, it tends to overestimate prey with long-retained hard parts, and underestimate easily digestible food (Baker et al., 2014; Buckland et al., 2017; dos Santos and Jobling, 1991).

In aquatic food webs, trophic relationships are increasingly frequently investigated through the analysis of fatty acid signatures of organisms (Czesny et al., 2011; Legeżyńska et al., 2014; Thiemann et al., 2008), because it provides a time-integrated depiction of a consumer's diet (Elsdon, 2010; Kirsch et al., 1998). It should be emphasised that in ecological studies, other biochemical techniques have also continued to develop to complement stomach content analysis (Nielsen et al., 2018). Considerable progress in feeding ecology also occurred owing to the integration of research concerning fatty acids and stable isotopes (Futia et al., 2021; Kelly and Scheibling, 2012). Fatty acids are compounds of lipids that occur in every body cell. Most of them, particularly polyunsaturated fatty acids (PUFA), have important physiological roles in the proper functioning of the organism, affecting its growth, reproduction, and survival (Parrish, 2009; Tocher, 2003). Most animals are not able to synthesize PUFA *de novo* at all, apart from elongating and desaturating them, but the rate of such conversion is very variable, and generally cannot meet the physiological requirements (Bell and Tocher, 2009; Tocher, 2003, 2010). Animals must therefore obtain PUFA primarily through diet.

The application of fatty acids trophic markers (FATM) in research on food-web relations is based on the assumption that particular species of prey have unique and identifiable fatty acid signatures that become incorporated into the consumer adipose tissue with little modification and in a predictable way (Budge et al., 2006; Dalsgaard et al., 2003). Such conclusions were primarily drawn based on research on the marine environment. Nevertheless, fatty acid analyses also proved useful in investigating the feeding habits of organisms in the freshwater environment, delineating spatial and temporal differences in diets both within and between species, and elucidating food web structure (Czesny et al., 2011; Käkälä et al., 2005; Thiemann et al., 2008). Moreover, this method is particularly promising due to the possibility of estimating the relative contributions of prey species in the diets of individual predators based on quantitative fatty acid signature analysis (QFASA). It has been applied in the investigation of the diet of seabirds and marine mammals (Iverson et al., 2004, 2007), and recently also fish (Happel et al., 2016a). It should be emphasised that although fatty acid compositions in consumers are undeniably influenced by diet, they can also be affected by other factors. According to the latest research, more species than previously believed are capable of modifying their dietary fatty acids

or even synthesising new fatty acids that had been considered to belong to essential fatty acids (Kabeya et al., 2018; Monroig and Kabeya, 2018). Furthermore, it has been proven that in addition to biosynthetic capacities, differences in the fatty acid composition may also vary with other intrinsic factors such as phylogeny and developmental and reproductive stages (Gladyshev et al., 2018; Maazouzi et al., 2011; Scharnweber et al., 2021; Szlinder-Richert et al., 2010). According to Chaguaceda et al. (2020), internal regulatory processes, associated with changes in physiological demands for PUFA over ontogeny, have similarly important effects on the fatty acid composition of fish as the diet. Research has also documented the effect of environmental conditions on the fatty acid composition in aquatic ecosystems (Galloway and Winder, 2015; Janer et al., 2007; Merad et al., 2018). Therefore, even though the importance of FATM has been demonstrated in numerous studies involving consumers from all trophic levels, including invertebrates, fish, birds, and mammals (Käkälä et al., 2005; Legeżyńska et al., 2014; Stowasser et al., 2009; Thiemann et al., 2008), the issue in the context of recent knowledge appears to be more complicated than was previously assumed.

The Baltic Sea, the largest brackish water body in the world, is becoming increasingly affected by climate change and anthropogenic pressures and all these stressors can lead to changes in the structure and functions of the ecosystem. The Baltic food web, from plankton communities via fish stocks to top predator populations, has undergone large changes during the few last decades (Casini et al., 2009; Möllmann et al., 2008, 2009; Wasmund and Uhlig, 2003). The increased pressure of many factors adversely affecting the quality of the environment and basic ecological processes particularly accumulates in estuarine and coastal environments (Airoldi and Beck, 2007; Collie et al., 2008). Like all over the world, also in the Baltic Sea, coastal waters play a crucial role in maintaining biodiversity in marine systems (Kraufvelin et al., 2018). However, the understanding of the structure and functioning of food webs in coastal waters such as estuaries and lagoons is a challenge because they are very productive and dynamic ecosystems (McLusky and Elliott, 2004).

The Vistula Lagoon is a coastal water body typical of the southern non-tidal Baltic Sea, where the structure of biocoenoses and ecological processes, including top-down regulations, are primarily determined by salinity (Kornijów, 2018). It provides a habitat for fish across various life stages, including spawning, juvenile development, feeding, and migration (Psuty and Wilkońska, 2009). It is one of the largest coastal lagoons in Europe which has been included in the NATURA 2000 protection program under the EU Habitats Directive and placed on the list of Baltic Sea Protection Areas. This study investigates the usefulness of fatty acid analysis in providing information on the diet and feeding habits of ichthyofauna in the Vistula Lagoon. Six fish species were selected due to their high abundance and importance in local fisheries, as well as their different feeding habits (omnivory, zooplanktivory, benthivory and piscivory). Special emphasis was placed on detailed fatty acid composition analysis to elucidate inter- and intraspecies differ-

ences, and reveal food sources and feeding behaviour discussed in the context of current knowledge of feeding ecology.

2. Material and methods

2.1. Study area

The study was carried out in the Vistula Lagoon, a large semi-enclosed shallow water body (area 838 km², mean depth 2.5 m, max. depth 5.2 m). The lagoon is separated from the Baltic Sea by the Vistula Spit. The inflow of sea-water is possible only through the Baltiysk Strait, contributing to uneven salinity along the basin (0.5–7.0 PSU). The area is influenced by both maritime and continental climates. The lagoon is very productive, with advanced eutrophic processes. The concentrations of total nitrogen and phosphorus in the water are high, reaching 1.1–4.4 mg dm⁻³ and 0.06–0.19 mg dm⁻³, respectively. The low water transparency usually oscillates around 40 cm (Kownacka et al. 2020; Nawrocka and Kobos, 2011). Despite highly advanced eutrophication and the accumulation of organic matter in the sediments, water oxygenation is high, even in winter under long-lasting ice cover (Glazunova and Polunina, 2013; Kornijów et al., 2020).

The near-shore littoral zone is primarily occupied by an intermittent belt of reed *Phragmites australis* (Cav.). In deeper waters, down to approximately 2 m, scattered patches of perfoliate pondweed *Potamogeton perfoliatus* L. and sago pondweed *Stuckenia pectinata* (L.) Börner occur (Kornijów, 2018; Pawlikowski and Kornijów, 2019). The phytoplankton of the lagoon is dominated by cyanobacteria, slightly more abundant in the middle than in the western basin. In the latter, a higher contribution is reached by diatoms and green algae (Kownacka et al., 2020). The zooplankton is dominated by Cladocera in the western, and Copepoda in the middle basin. Cladocera include numerous filtrators, as well as predatory *Leptodora kindtii*. Rotifera are relatively more abundant in the western basin (Paturej and Gutkowska, 2015; Paturej et al., 2017). Due to the brackish water conditions, the zoobenthos lacks many freshwater taxa, e.g. insects (Odonata, Ephemeroptera, Trichoptera) and gastropods. In terms of density, macroinvertebrates are dominated by detritivorous Tubificinae and larvae of Chironomidae. In the biomass, two alien species prevail, namely a bivalve clam *Rangia cuneata* and a polychaete *Marenzelleria* sp. (Kornijów, 2018). The ichthyofauna is mostly composed of freshwater species (Kornijów, 2018; Psuty and Wilkońska 2009). Marine fish such as flounder (*Platichthys flesus*), turbot (*Scophthalmus maximus*), and Atlantic herring (*Clupea harengus*) occur in the lagoon only periodically. Non-piscivores (except for periodically occurring herring) are dominated by ruffe (*Gymnocephalus cernua*), roach (*Rutilus rutilus*), bleak (*Alburnus alburnus*), European smelt (*Osmerus operlanus*), and silver bream (*Blicca bjoerkna*). Piscivores are dominated by pikeperch and perch.

2.2. Sampling and laboratory analyses

Specimens of several most frequently fished fish species, such as sichel (*Pelecus cultratus*), pikeperch (*Sander lucioperca*), European perch (*Perca fluviatilis*), European eel (*Anguilla anguilla*), bream (*Abramis brama*), and roach (*Rutilus rutilus*), were collected from May to June 2016 from the Polish part of the Vistula Lagoon. The fish were measured (wet mass and total length) and frozen immediately after collection. Before analysis, they were filleted and skinned. Then, the muscle tissue from each individual was homogenised and freeze-dried.

Fatty acids were analysed in the total lipid fraction. Lipids were extracted with a mixture of dichloromethane:methanol (2:1 v/v), according to the procedure by Folch et al. (1957). The dichloromethane phase containing total lipids was collected and reduced to dryness under a stream of nitrogen. Fatty acid methyl esters (FAME) were prepared following the methods by Usydus et al. (2011). Briefly, 0.1 g of the extracted lipid was dissolved in 1.6 ml of 2 M methanolic potassium hydroxide solution, and shaken vigorously. The solution was heated, and after its cooling, 3.2 ml of 4% methanolic solution of hydrochloric acid was added. The samples were reheated. After their cooling, 1.6 ml of isoctane was added. Then the solution was vortexed and adjusted to a volume of 10 ml with a saturated solution of sodium chloride. Anhydrous sodium sulphate was added to dry the extracts. The resultant solution of FAME on the top layer was diluted with methanol in a proportion of 1:4 v/v, and was subject to final determination. FAME were determined using gas chromatography equipped with a flame ionisation detector (GC-FID). The column used was a Restek Rt-2560 (100 m x 0.25 mm x 0.2 µm film thickness). The chromatography conditions were as follows: split injection; split ratio – 100:1; injection volume – 2 µm; carrier gas flowing at 1.1 ml min⁻¹ – helium; injector port temperature – 250°C; FID temperature – 260°C; oven temperature – initial oven temperature 140°C held for 2 min, then increased to 225°C at a rate of 2°C min⁻¹ and held for 10 min, followed by an increase to 240°C at a rate of 40°C min⁻¹ and held for 10 min. The instrumental analytical precision was determined by 5 replicate injections of the standard, which gave coefficients of variation in the response value in the range of 0.2–2.1%. The identity of several FAME was confirmed by gas chromatography-mass spectrometry (GC-MS). The same column and temperature programme as described above were used. The interface to the mass analyzer was maintained at 240°C, and the mass analyzer used a 70-eV ionization potential, and scanned over a mass range of 50–500 m/z. The individual FAME were identified by comparison of retention times with PUFA1, PUFA3, and a 37-component FAME mixture supplied by Supelco, and confirmed by comparing our mass spectrum with that from the American Oil Chemists' Society Lipid Library (<http://lipidhome.co.uk/ms/masspec.html>). Results for each FAME were presented in relative units, as percentages of total fatty acids, throughout the paper. The fatty acids were grouped into saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids

(PUFA). The latter were further divided into sums of n-3 PUFA and n-6 PUFA.

2.3. Statistical analysis

We detected a total of 37 fatty acids in fish tissue, but fatty acids that did not exceed 0.5% of total fatty acid in at least one fish sample were excluded from all statistical analyses. The remaining ones were re-calculated to 100%. To explore overall patterns in fatty acid data, we performed multivariate analyses. Multidimensional scaling (MDS) analysis seeks to capture compositional similarities in the fewest dimensional space. We used MDS to illustrate the relations between particular specimens of the studied species. Principal Component Analysis (PCA) was performed to identify fatty acids that contributed the most to the observed inter- and intraspecific differences. Principal components (PC) were extracted based on eigenvalues greater than 1. Variables with a loading factor of >0.6 were considered significant. The relationships between fish length and fatty acids were examined by means of the Spearman rank correlation test. Statistical analyses were performed using the Statistica software version 10.0 (StatSoft, 2011).

3. Results

The analysis involved twenty-one fatty acids quantified in all species with a percentage in the sum of all identified fatty acids greater than 0.5%. The selected fatty acids constituted 97.9% to 99.5% (average 98.8%) of the total of all detected fatty acids. The summary information for the tested samples and fatty acids is presented in Table 1. For most of the studied species, a higher proportion of PUFA than that of MUFA and SFA was generally observed, except for bream and European eel in which MUFA predominated (Table 1). The percentages of PUFA in the tested species varied from 19.5% in eel to more than 50% in perch and pikeperch, while the percentage of MUFA ranged from 13.9% in perch to 52.8% in eel. SFA contents were comparable in all the studied species, and were within a fairly narrow range from 22.8% to 29.2%. Another common feature shown for all the studied species was a significant predominance of fatty acids from the n-3 PUFA family (15.0–51.3% of total fatty acids) over fatty acids from the n-6 PUFA family (4.5–10.1% of total fatty acids). The average value of the n-3/n-6 PUFA ratio differed between the species, and reached from 2.2 in bream to 5.4 in perch. The value was broadly variable within fish. It ranged 3.0–9.0, 1.0–6.2, 0.4–4.3, 2.5–5.9, and 3.1–4.8 in pikeperch and perch, roach, bream, eel and sichel, respectively.

3.1. Interspecies variation in fatty acid compositions

The studied species differed in terms of the highest abundance of particular fatty acids. In perch and pikeperch, docosahexaenoic acid (22:6n-3, DHA) reached the highest percentage. In contrast, the fatty acid signatures in eel, bream, and sichel were dominated by 18:1n-9, while in

the lipid of roach, 16:0 predominated. The analysis of the data from Table 1 evidently shows that the most apparent differences found between the studied species were the mean percentages of the following six out of 21 quantified fatty acids: 16:0 (15.6–20.4%), 16:1 (3.8–9.8%), 18:1n-9 (6.0–37.2%), arachidonic acid (20:4n-6, ARA) (1.5–7.3%), eicosapentaenoic acid (20:5n-3, EPA) (2.9–9.3%) and DHA (4.2–35.0%).

MDS analysis permitted illustrating the relationship between the studied fish based on their fatty acid signatures (Figure 1). The samples were grouped by species in the diagram. Samples representing perch and pikeperch, eel, and sichel evidently formed compact clusters, relatively distant from each other, while samples representing roach and bream formed a common group, quite extended in the diagram, suggesting greater intraspecific variability for these two latter species. The position of the groups corresponding to each species in the diagram can be to some extent explained by the type of their diet. Samples representing two piscivorous species, namely perch and pikeperch, were located on the left side of the diagram in close proximity to each other, and could be distinguished from other species by negative values on the MDS1 axis, while most samples of the other species showed positive values on the MDS1 axis. Roach and bream, whose dietary composition is very diverse and includes both benthic and pelagic prey, commonly formed a large cluster stretching from the middle to the right part of the diagram. Samples that formed that cluster exhibited values on the MDS2 axis ranging from -0.9 to +2.5. Samples of zooplanktivorous/facultative piscivorous sichel were located close to roach and bream, while samples of opportunistic carnivorous species – eel were relatively distant from the other studied species, and characterised by the lowest values on the MDS2 axis. It is also worth emphasising that although in Figure 1 pikeperch and perch form a single cluster evidently separated from the remaining species, the MDS2 axis to a certain extent separates specimens of both species in terms of their length. Pikeperch with a body length from 31 cm to 50 cm reached higher values on the MDS2 axis (from 0.3 to 0.9) than 2 specimens of pikeperch with a body length of 53 cm and 56 cm (from -0.5 to -0.1). In the case of perch, all specimens with a body length below 27 cm were characterised by low values on the MDS2 axis (from -0.5 to 0.4), whereas the largest specimens of perch (>27 cm) showed positive and negative values on the MDS2 axis.

The principal component analysis (PCA) was used to identify fatty acids that contributed the most to the observed differences between the studied fish species (Figure 2). Overall, this PCA explained 85.6% of total variability using 5 principal components. The first principal component (PC1) explained 44.4% of the total variance. This PC was strongly negatively correlated with ARA, EPA, and DHA, and strongly positively correlated with 14:0, 16:1, 18:1n-9. On the one hand, this factor distinguished pikeperch and perch among the studied species due to the high percentage of ARA, EPA, and DHA in their tissues. On the other hand, a high percentage of 14:0 and monounsaturated fatty acids were a characteristic feature of eel. The second principal component (PC2) accounted for 16.7% of the total variance, and was negatively associated with the majority of SFA, particularly 16:0, 18:0, and 20:0. Samples representing bream, roach

Table 1 Morphological characteristics (mean, min-max) and fatty acid composition (mean±SD) in fish from the Vistula Lagoon.

	sichel <i>Pelecus cultratus</i>	pikeperch <i>Sander lucioperca</i>	perch <i>Perca fluviatilis</i>	bream <i>Abramis brama</i>	roach <i>Rutilus rutilus</i>	eel <i>Anguilla anguilla</i>
n	31	16	38	53	34	12
length [cm]	35.4	40.9	22.6	30.2	22.7	58.0
	30.5–42.0	31.0–56.0	16.5–31.0	13.0–49.5	15.0–31.0	56.0–60.0
mass [g]	263.6	606.3	154.0	444.5	156.5	342.6
	163.3–440.5	250.0–1433.5	51.5–329.4	25.9–1521.4	36.7–431.3	284.9–398.5
fatty acid [%]						
14:0	1.78±0.28	1.14±0.14	1.09±0.24	2.17±0.48	1.90±0.50	3.91±0.29
15:0	0.33±0.04	0.43±0.05	0.37±0.06	0.46±0.09	0.54±0.14	0.36±0.06
16:0	15.55±0.68	20.01±1.47	18.44±2.27	20.43±3.98	19.83±2.30	18.45±0.69
17:0	0.67±0.07	0.81±0.14	0.64±0.13	0.82±0.15	0.90±0.20	0.58±0.11
18:0	4.42±0.48	6.15±0.97	4.34±0.58	5.33±1.27	4.71±0.60	4.22±0.29
20:0	0.19±0.02	0.24±0.08	0.19±0.05	0.27±0.08	0.20±0.04	0.14±0.02
SFA	22.76±0.93	28.54±1.84	24.89±2.82	29.22±5.18	27.87±3.10	27.52 ±0.86
16:1	8.58±1.48	4.58±0.77	3.80±1.35	9.81±2.24	9.40±2.76	9.26±0.61
18:1n-9	21.01±2.58	7.39±0.84	6.03±1.50	23.50±4.74	16.92±4.36	37.17±1.41
18:1n-7	5.50±0.41	4.01±0.50	3.28±0.61	5.17±0.72	4.95±0.96	4.37±0.22
20:1n-11	0.27±0.11	0.14±0.05	0.09±0.05	1.12±0.72	1.06±0.64	0.65±0.37
20:1n-9	1.57±0.15	0.40±0.05	0.40±0.10	0.93±0.31	1.12±0.27	1.27±0.27
24:1	0.14±0.02	0.40±0.07	0.29±0.11	0.13±0.11	0.11±0.04	0.07±0.02
MUFA	37.06±3.89	16.93±1.46	13.89±3.40	40.66±7.06	33.57±7.71	52.80±1.34
18:2n-6 (LA)	2.83±0.54	2.02±0.31	2.04±0.68	4.03±1.29	3.23±1.72	2.25±0.72
18:3n-3 (ALA)	1.62±0.21	1.17±0.14	0.98±0.22	2.38±0.66	2.70±0.84	1.83±0.20
20:2n-6	0.85±0.15	0.50±0.10	0.39±0.07	1.08±0.26	0.95±0.16	0.60±0.19
20:3n-6	0.26±0.04	0.24±0.07	0.20±0.04	0.43±0.13	0.41±0.09	0.22±0.02
20:3n-3	0.55±0.07	0.35±0.07	0.27±0.04	0.60±0.19	0.54±0.13	0.43±0.07
20:4n-6 (ARA)	3.99±0.64	7.34±1.54	7.16±1.00	3.77±1.67	4.45±1.17	1.45±0.26
20:5n-3 (EPA)	6.97±0.65	8.35±0.61	9.32±0.99	5.03±2.14	5.72±1.43	2.86±0.62
22:5n-3	4.12±0.41	6.37±1.08	5.72±1.48	3.79±1.65	5.10±1.13	5.68±0.99
22:6n-3 (DHA)	18.78±2.62	27.94±3.27	34.96±6.12	8.74±5.07	15.27±8.28	4.23±1.12
PUFA	39.99±3.49	54.29±2.35	61.03±6.05	29.85±9.73	38.36±9.88	19.54±1.60
n-3 PUFA	32.05±3.04	44.18±3.10	51.25±6.84	20.54±8.44	29.32±9.56	15.02±1.63
n-6 PUFA	7.94±0.88	10.11±1.74	9.78±1.48	9.31±2.07	9.04±1.58	4.52±0.73

SFA – Saturated fatty acids; MUFA – Monounsaturated fatty acids; PUFA – Polyunsaturated fatty acids; LA – Linoleic acid; ALA – α -linolenic acid; ARA – Arachidonic acid; EPA – Eicosapentaenoic acid; DHA – Docosahexaenoic acid.

and perch adopted wide ranges of scores. The next principal component (PC3) (13.4% of total variance) had high positive factor loadings for 15:0 and 17:0 among SFA, 20:1 n-11 among MUFA, and α -linolenic acid (18:3n-3, ALA), 20:2n-6 and 20:3n-6 among PUFA. Roach and bream were grouped together in this ordination. The fourth principal component (PC4) accounted for 6.3% of the total variance, and linoleic acid (18:2n-6, LA) had the largest effect on that PC. The last principal component (PC5), explaining 4.8% of the total variance, was marked by high loading on 20:1n-9. The last two principal components distinguished mainly bream and sichel, respectively.

Very wide ranges of scores obtained for some principal components suggest high intra-species variability of the

fatty acid composition in certain cases (Figure 2). Due to this, separate PCA analyses were performed for each species to investigate the intraspecific variability in more detail.

3.2. Intra-species variation in fatty acid compositions

The PCA conducted for each species separately indicated differences in fatty acid signatures between different size classes of perch, bream, and roach (Figure 3). For perch, the first two components explained 56.0% of the total variance, whereas the first component explained 41.6% of the total

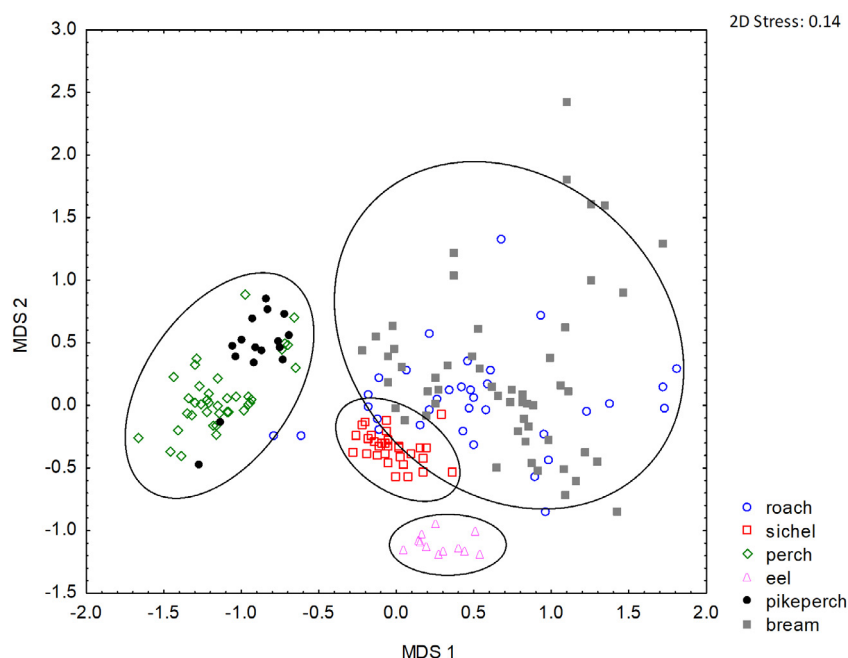


Figure 1 Multidimensional scaling (MDS) plot for all studied fish species. The analysis was based on 21 fatty acids with a percentage higher than 0.5%. The data were standardised to equal 100% prior to analysis. Each point represents an individual fish.

variance. PCA analysis (Figure 3A) confirmed the conclusions previously drawn in the interpretation of the MDS analysis. The samples, represented by smallest size class perch (16.5–19 cm), reached high positive values on the PC2 axis, whereas the samples representing perch in the medium size class (21–26 cm) largely showed negative values on the PC2 axis. The PC2 axis was positively correlated with 20:0, and negatively correlated with 20:1n-11. All these samples (small- and medium-size classes) also showed negative values on the PC1 axis which was strongly negatively correlated with DHA, and strongly positively correlated particularly with 16:0, 18:1n-9, and 18:1n-7. Furthermore, PC 1 separated the largest specimens (>27 cm) into two groups. The analysis of individual fatty acids for their relationship with perch length indicated a statistically significant relationship between these variables ($p < 0.05$, $0.46 < R_s < 0.71$). With an increase in body length, the percentage of EPA and DHA in the muscle tissue decreased, and the percentage of 16:0, 16:1, 18:1n-9, 18:1n-7, 20:1n-9, and 18:2n-6 increased.

According to PCA analyses, the largest size classes of bream and roach were closely grouped, while the variation was high for specimens measuring less than 24 cm (Figure 3B,C). For bream, the first (PC1) and second principal components (PC2) explained 37.7 and 26.3% of the total variance, respectively (Figure 3B). The samples represented by bream with a body length of more than 24 cm largely showed negative values on the PC1 axis which had high negative factor loadings primarily for EPA and 22:5n-3, and high positive factor loadings for 16:0, 18:0, 20:0, and 24:1. Specimens smaller than 24 cm formed two groups that showed both positive and negative values on the PC1 and PC2 axis. PC2 was strongly positively correlated with ARA, and strongly negatively correlated with 14:0, 15:0, and 16:1. For bream, the statistically significant correlation between individual fatty acids and fish length was indi-

cated ($p < 0.05$, $0.44 < R_s < 0.82$). With an increase in length in bream, the percentage of 15:0, 20:1n-11, 18:3n-3, 20:2n-6, 20:3n-3 and EPA increased, and the percentage of 16:0, 18:0, 20:0, and 24:1 decreased.

For roach, the PC1 axis accounted for 34.3% of the total variance, and showed a strong negative correlation with PUFA, mainly DHA and EPA, while SFA and MUFA were positively correlated with PC1. PC2 (22.3% of total variance) was strongly positively correlated with 20:1n-11 and 22:5n-3, and negatively correlated with 18:2n-6. Moreover, 15:0, 17:0, 18:1n-7, 20:1n-11, and 18:3n-3 were positively correlated with both PC1 and PC2. Roach from different size classes were separated as presented in Figure 3C. Furthermore, in roach, like in bream, a positive length-related trend with 15:0, 20:1n-11, and 18:3n-3 was observed ($p < 0.05$, $0.35 < R_s < 0.69$). In roach, a similar positive relationship was also found for 18:1n-7 and 20:1n-9, while for 18:2n-6, a negative length-related trend was found ($p < 0.05$, $0.49 < R_s < 0.65$).

4. Discussion

Our study demonstrated that the majority of the investigated fish species could be distinguished by their fatty acid signatures. The applied multivariate analyses permitted grouping of the studied fish samples according to their fatty acid signatures into four clusters (Figure 1). Based on the current knowledge of the ecology of the studied species, the obtained pattern can be largely explained by the diet characteristic of particular species. A deeper analysis of the data additionally allows for the identification of factors other than the diet that may affect fatty acids composition in fish. As shown in Figure 1, cluster 1 collected samples representing two predatory species of the Percidae

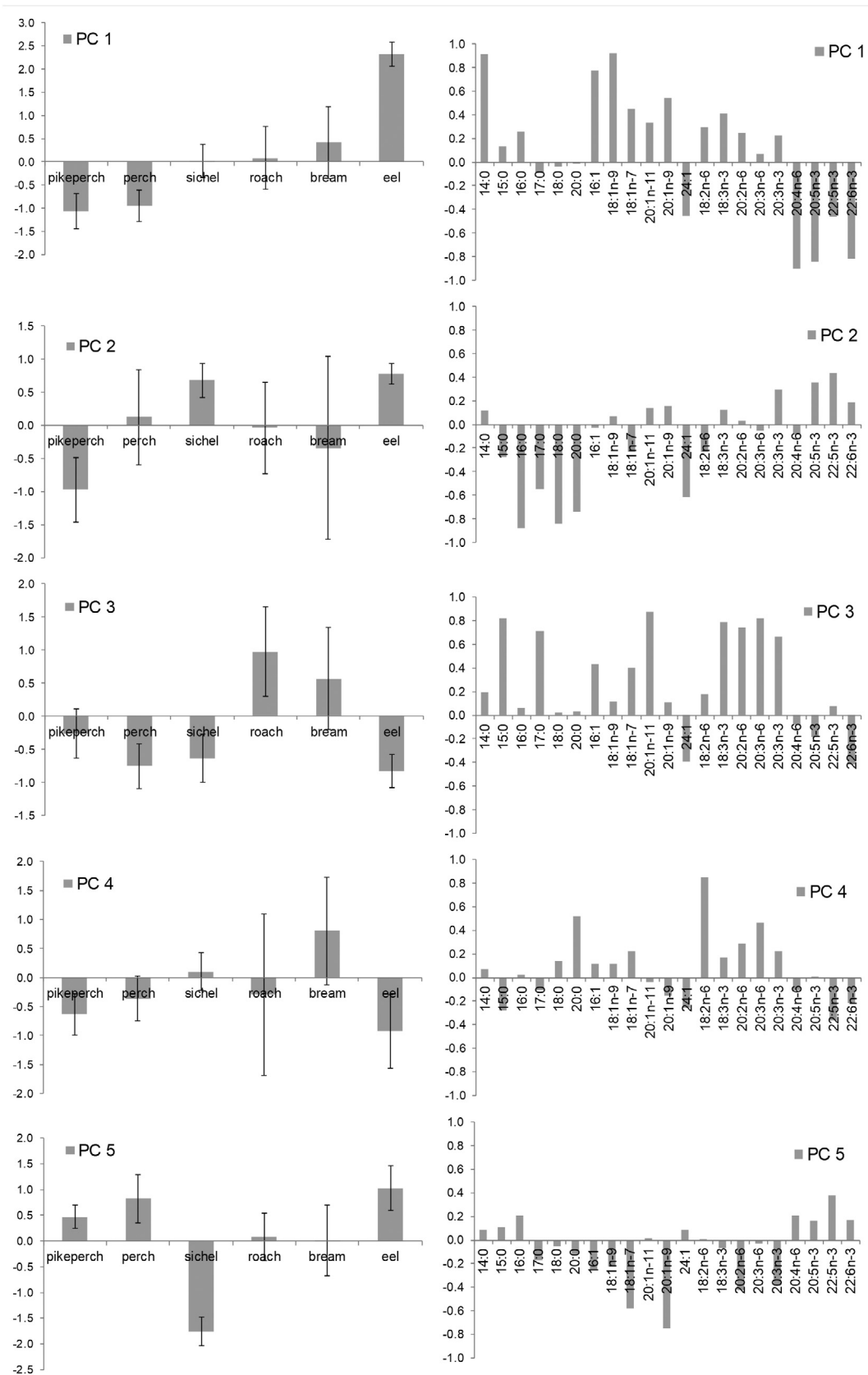


Figure 2 Principal component analysis (PCA) of fatty acids in the muscle tissue for all studied fish species. Diagrams of mean scores (to the left) and factor loadings (to the right) for the defined principal components. The PCA employed normalised Varimax rotation.

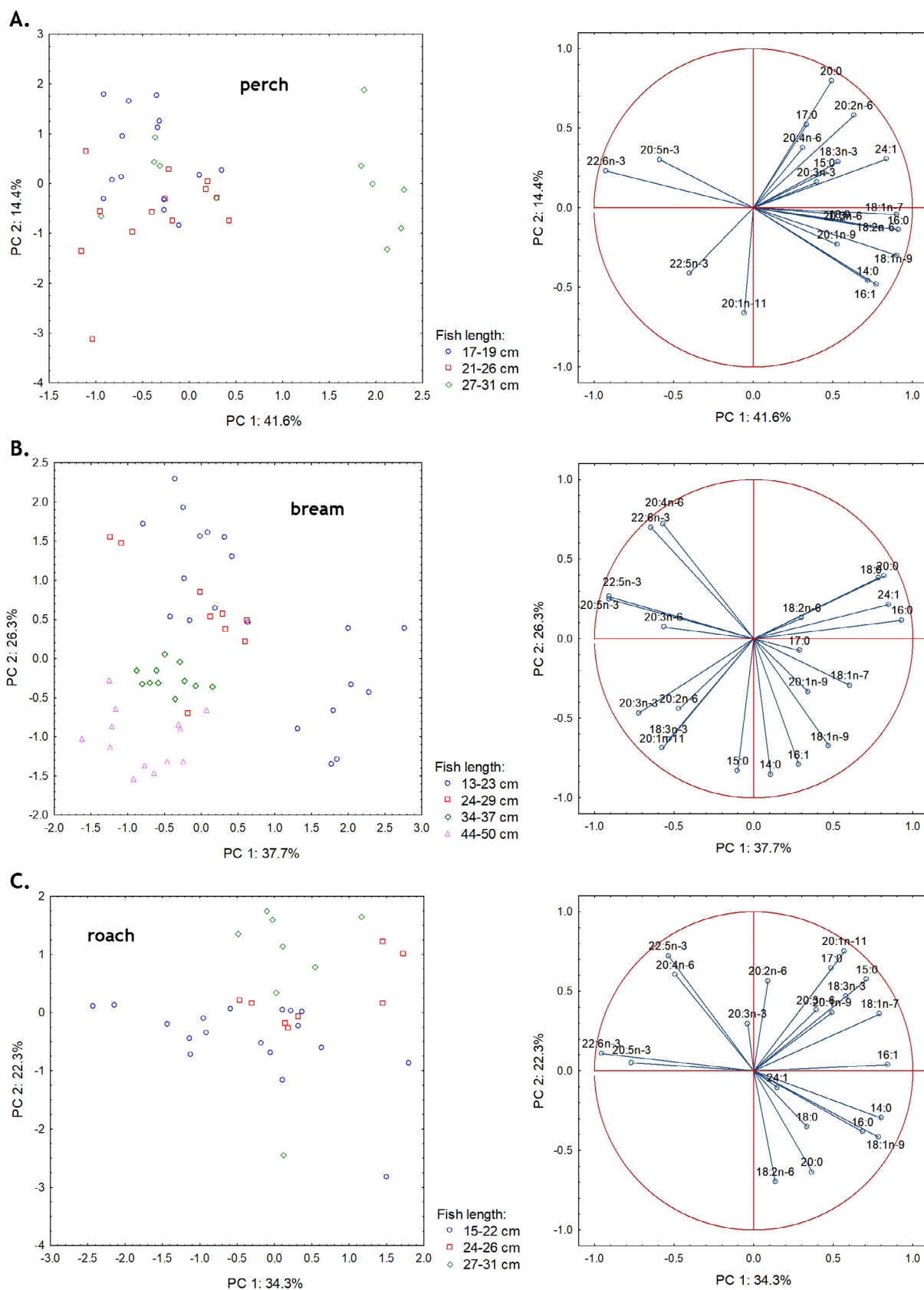


Figure 3 Scores (to the left) and the factor loadings (to the right) of the first two principal components in a PCA model built based on fatty acid compositions in the muscle of perch (A), bream (B) and roach (C).

family: perch and pikeperch. Samples representing species from the Cyprinidae family formed two closely located clusters, cluster 2 (bream and roach) and cluster 3 (sichel), while cluster 4 covered samples representing individuals of European eel. The differences in fatty acid signatures between the studied species are largely attributable to differences in DHA, EPA, ARA, and 18:1n-9. Samples forming cluster 1 were characterised by high proportions of DHA, EPA, and ARA. Samples involved in clusters 2 and 3 were in turn characterised by a much lower content of those fatty acids, but also by a distinctly higher proportion of 18:1n-9 in comparison to predatory species. Samples from cluster 4 had the lowest proportion of DHA, EPA, and ARA among all analysed samples, and the highest proportion of 18:1n-9 and 14:0.

The feeding ecology of pikeperch and perch assigned to cluster 1 in the current study are well known (Demchuk et al., 2021; Hansson et al., 1997; Hjelm et al., 2000; Lehtonen et al., 1996; Mustamaki et al., 2014). Pikeperch can be classified as an obligatory piscivorous, while perch is considered as an omnivorous predator. Pikeperch usually becomes piscivorous during the first year of life, while perch undergoes ontogenetic diet shifts from feeding on zooplankton, through benthic invertebrates, to the piscivory phase. DHA, EPA, and ARA occurring in the lipid of their muscle tissue in a significant proportion belong to highly unsaturated fatty acids (HUFA) with high nutritional value and high potential to affect consumer fitness through their impact on many vital processes (Tocher, 2003). Dietary deficiencies of these fatty acids can have serious consequences, including inhibited growth, limited ability to reproduce, and increased incidences of disease and metabolic disorders. Probably due to the physiological importance of these compounds, they are retained in aquatic food webs and are effectively transferred to higher trophic levels (Gladyshev et al., 2011; Koussoroplis et al., 2011; Lau et al., 2012; Strandberg et al., 2015). According to the current knowledge, DHA is selectively and highly accumulated over other PUFA in fish. Investigating the retention of PUFA in different fish taxa from streams, Guo et al. (2017) indicated that Perciformes had higher proportions of DHA, EPA, and ARA than fish from the Cyprinidae family, but significant differences were only found in DHA. A similar result was obtained for fish from the Vistula Lagoon in the current study. Sushchik et al. (2017) also confirmed a higher proportion of DHA in piscivorous fish, including perch and pike, in comparison to Cyprinidae (roach and bream). The cited study also evidenced, however, that the percentage of EPA in Cyprinidae can be higher than in Percidae, as was the case for fish from the Krasnoyarsk Reservoir (Siberia, Russia).

The differences in fatty acid signatures between perch below and above 27 cm length indicated in the current study are intriguing. This result suggests that in the Vistula Lagoon, perch start feeding on similar prey (fish) as pikeperch only after reaching 27 cm body length. Our results also suggest that even after reaching a certain size threshold that predisposes perch to piscivory, it may still feed on invertebrates. This is suggested by the dispersal of perch samples larger than 27 cm observed in the MDS and PCA analyses (Figures 1, 3A). A similar conclusion was drawn by Mustamaki et al. (2014). According to the authors, irrespec-

tive of its size, perch feeds both on fish and invertebrates in the northern Baltic Proper. The PCA analysis also revealed high variability of fatty acid signatures in perch from different size classes (Figure 3A). Perch individuals included in the current study showed a significant variation in body length (16.5–31.0 cm). Individuals of small and medium size classes generally showed a higher percentage of n-3 PUFA, particularly DHA, and a lower percentage of SFA (i.e. 16:0) and MUFA (i.e. 16:1, 18:1-9, 18:1n-7) than the largest size class. Medium-sized perch had higher proportions of 20:1n-11 and 22:5n-3 than the remaining individuals. Although the determination of perch's detailed diet based on the analysis of fatty acid signatures is difficult due to the lack of fatty acid signatures of putative prey species, certain conclusions can be drawn. The results suggest that perch become piscivorous after reaching a body size of 27 cm, and below that size feeds on various invertebrates. According to Chaguaceda et al. (2020), piscivorous perch in comparison to planktivorous and benthivorous ones is characterised by higher proportions of 16:1 and 18:1n-9, and a lower proportion of EPA. In our study, the piscivory of perch was additionally confirmed by the MSD analysis in which perch with high proportions of 16:1 and 18:1n-9, and a low proportion of EPA was identified together with pikeperch (Figure 1). The literature (Czesny et al., 2011; Happel et al., 2015; Kornijów et al., 2016; Lau et al. 2012; Makhutova et al., 2011) and our results suggest that the small-size class of perch relies more on pelagic crustaceans, while the medium-size class, first of all, depends on benthic invertebrates. The difference between perch of small- and medium-size classes was primarily driven by differences in proportions of 20:1n-11, DHA and EPA (Figure 3A). EPA and DHA are commonly found to be prevalent in pelagic sources, while 20:1n-11 is frequently detected only in oligochaetes and bivalves, typical benthic invertebrates (Kornijów et al., 2021; Lau et al. 2012; Makhutova et al., 2011). Medium-sized perch also showed a higher proportion of 16:1, and lower proportions of n-3 PUFA in comparison to the smallest perch, which additionally supports the thesis on the influence of benthic feeding (Czesny et al., 2011; Happel et al., 2015).

On the other hand, according to Chaguaceda et al. (2020), in perch, the diet explains only a limited part of fatty acid variability. Equally important are internal regulatory processes. A decrease in the proportion of EPA and DHA, and an increase in MUFA in the muscle tissue during fish growth were also reported for other fish species, and are probably related to changes in the energy requirements throughout the life cycle (Maazouzi et al., 2011; Makhutova and Stoyanov, 2021; Tocher, 2010). Another factor that may be responsible for changes in fatty acid profiles is the ability of some species to biosynthesise HUFA through bioconversion. In fish, like in all vertebrates, C18 PUFA such as ALA and LA cannot be synthesised *de novo* and must come from the diet. However, some fish species can convert dietary ALA and LA to their biologically active long-chain derivatives, including n-3 EPA and DHA, and n-6 ARA. The ability of fish to elongate and desaturate C18 precursors varies greatly between species, and has been assumed to be habitat- and trophic level dependent (Tocher, 2003; Trushenski and Rombenso, 2020). It is generally accepted that most marine fishes and many carnivorous fishes are not

capable of such biochemical conversion at a physiologically significant rate. In contrast, freshwater fish, mainly herbivorous and omnivorous, are capable of meeting the physiological demand for HUFA through such biosynthetic capacity. Nevertheless, knowledge of the bioconversion capabilities in fish is still limited, particularly for wild fish, and therefore further research is required. The latest study on perch from the natural environment showed that EPA levels in the muscle tissue corresponded with those in consumed resources, but a mismatch was recorded between ARA and DHA proportions in the consumer muscle tissue compared to their resources in the diet (Scharnweber et al., 2021). Sawyer et al. (2016) showed, based on the mass-balance model, that in the case of yellow perch (*Perca flavescens*), the main source of EPA and ARA was diet, while DHA came primarily from bioconversion. Henrotte et al. (2011) demonstrated that Eurasian perch was able to elongate and desaturate ALA into DHA but showed a rather limited capacity for the elongation of LA to ARA. Moreover, they indicated the dependency of bioconversion efficiency on ontogeny. To sum up, the current study does not unequivocally point to specific factors responsible for the observed change in fatty acid profiles in perch. However, they suggest that in certain species, EPA is a better indicator of diet than DHA.

As previously mentioned, fish from the Cyprinidae family, namely sichel, bream, and roach were characterised by significantly lower proportions of DHA, EPA, and ARA, and a higher proportion of 18:1n-9 than predatory species. Additionally, sichel had the highest percentage of 20:1n-9 among the studied fish species, and a lower percentage of 16:0 and 20:1n-11 compared to bream and roach. Higher content of 20:1n-9 in Cyprinidae indicates zooplankton in their diet, since monounsaturated fatty acids with 20 and 22 carbon atoms are particularly abundant specifically in Calanoid copepods, and have been recommended as zooplankton markers (Dalsgaard et al., 2003; Kelly and Scheibling, 2012). Specifically herbivorous copepods are able to synthesise *de novo* considerable amounts of 20:1n-9 and 22:1n-11 fatty acids, which typically accumulate in wax esters as a long-term energy reserve (Lee et al., 2006). In the case of the tested species, the content of 22:1 did not exceed 0.2% of total fatty acids, while 20:1n-9 showed relatively high content in sichel (1.1–1.8%). This result is in agreement with some studies from the Baltic Sea region (Keinanen et al., 2017; Lind et al., 2018), where a higher ratio of 20:1 to 22:1 was detected in other zooplanktivorous species such as herring *Clupea harengus membras* and sprat *Sprattus sprattus* which predominantly consumed small-sized copepods such as *Temora longicornis*, *Eurytemora affinis*, and *Acartia* spp. (Ojaveer et al., 2018). Our results are also consistent with a previous study based on stomach content analysis which classified sichel as a zooplanktivore/facultative piscivore (Specziár and Rezsú, 2009; Stolarski, 1995). Total lengths of sichel studied in the Vistula Lagoon ranged from 30.5 cm to 42 cm. According to the literature, in the Vistula Lagoon sichel larger than 20 cm starts to feed on fish and becomes a facultative predator (Stolarski, 1995). Its prey is primarily small pelagic fish such as smelt, as well as all juvenile fish, which in turn rely on zooplankton. It is important to emphasise that zooplankton plays a key role in linking food webs, serving as the main energy pathway from primary producers to higher trophic level organisms. Copepods are the main

component of zooplankton by biomass in the Vistula Lagoon, with a distinct spring peak largely accounted for by *Eurytemora affinis* (Dmitrieva and Semenova, 2012). This is of particular importance for larval survival and subsequent recruitment of fish because copepods have a high DHA content compared to other crustacean zooplankton (Persson and Vrede, 2006). Especially, larvae and juveniles require a large amount of DHA for proper development and ultimately survival (Bell et al., 1995; Ishizaki et al., 2001; Mourente et al., 1991).

Samples representing bream and roach formed one scattered cluster (Fig. 1). This confirms the general opinion that the diet of both species, feeding mainly on zooplankton and macroinvertebrates is diverse and partly overlaps (Lammens and Hoogenboezem, 1991; Nagelkerke and Sibbing, 1996; Specziár et al., 1997). Roach is considered one of the most successful generalists, feeding on zooplankton and macroinvertebrates, including molluscs and live or dead plant material (Demchuk et al., 2021; Kornijów et al., 2005; Specziár and Rezsú, 2009). Compared with roach, bream is more dependent on the food of animal origin, with a preference for soft-bodied macroinvertebrates buried in sediments (Lammens et al., 1985; Nagelkerke and Sibbing, 1996). It is also worth emphasising that in the case of both fish species from the Vistula Lagoon, the highest variability of fatty acid signatures particularly concerned specimens of small size classes (roach: 15–22 cm; bream: 13–23 cm; Figure 3B, C). The variation observed between individuals of different size classes was primarily related to the percentage of HUFA and 20:1n-11, 18:3n-3. The proportion of 20:1n-11 and 18:3n-3 increased with an increase in body length in both species. While for roach, the proportion of 15:0, 17:0 and 18:1n-7, used as tracers for the contribution of bacteria (Kelly and Scheibling, 2012), also increased. This suggests that the diet of bream and roach was rich in bivalves and/or oligochaetes, as previously 20:1n-11 has been detected only in these invertebrates (Makhutova et al., 2011). According to the literature, molluscs are the most important constituent of the roach diet (Specziár and Rezsú, 2009; Specziár et al., 1997). It is related to the structure and functioning of their feeding system. Molluscs have also been found in the digestive tracts of bream, although it is believed that roach can switch to feed on molluscs faster than bream (Nagelkerke and Sibbing, 1996; Prejs et al., 1990). The reason is that bream is able to penetrate the sediment to a greater depth than roach (Lammens et al., 1985; Persson and Brönmark, 2002), and is, therefore, more efficient at feeding on benthic organisms associated with the sediments.

Although the analysis of fatty acid signatures in roach and bream allows for tracing some shifts in their diet composition in relation to their size, it would be very difficult to precisely conclude the detailed composition of their diet. As previously indicated, the use of FATM to study benthic food web interactions is very complicated (Kelly and Scheibling, 2012). Moreover, contrary to what has been believed for decades, recent studies provide evidence that not only primary producers but also a wide range of invertebrates possess genes involved in the biosynthetic pathways of PUFA, including *de novo* biosynthesis of C18 PUFA (Kabeya et al., 2018; Monroig and Kabeya, 2018). It has been recently confirmed that various aquatic inver-

tebrates such as annelids as well as molluscs and arthropods which dominate benthic habitats can produce PUFA endogenously, which presents challenges when reconstructing dietary links. The bottom fauna of the Vistula Lagoon is not abundant in terms of a number of species. The major components of zoobenthos are larvae of Chironomidae and Oligochaeta (Ezhova et al., 2005; Kornijów et al., 2021). Over the last few decades, *Marenzelleria* sp., belonging to the group of Polychaetes, has also been found in large numbers in the Vistula Lagoon (Żmudziński, 1996). Nonetheless, probably due to the ability of these invertebrates to burrow deep in the bottom sediments, they do not constitute food available for benthivorous fish (Golubkov et al., 2021; Żmudziński, 1996). The most numerous taxa among molluscs are bivalves, dominated by two alien species – *Dreissena polymorpha* and *Rangia cuneata* (Kornijów, 2018). Fatty acid data of Oligochaeta are scarce, whereas data regarding Chironomidae and *Dreissena polymorpha* are more abundant. According to the literature, the main difference between bivalves and Chironomidae and Oligochaeta is the fact that the latter are almost devoid of DHA, whereas in bivalves it occurs in considerably higher proportion (Budge et al., 2001; Czesny et al., 2011; Makhutova et al., 2011). The presence of 20:1n-11 is a characteristic feature of Oligochaeta and bivalves while it is not recorded in Chironomidae. Moreover, in comparison to Chironomidae, Oligochaeta show a lower percentage of 16:1, LA, ALA and EPA (Goedkoop et al., 2000; Makhutova et al., 2011; Sushchik et al., 2006). It is worth emphasising that, based on stomach content analysis, the importance of oligochaetes in fish diets is often underestimated due to their high rate of digestion and therefore frequently impossible identification of their remains in the digestive contents (Bouguenec and Giani, 1989; Wiśniewski, 1978). Bivalves can be in turn overestimated due to the resistance of their shell to the digestive processes. This research, however, does not permit detailed determination of the prey of roach and bream, because probably the key importance lies in the ability to modify fatty acids by these fish species (Galloway and Budge, 2020). According to the literature, larvae and pupae of Chironomidae are an important component of the diet of benthivorous and omnivorous fish (Filuk and Żmudziński, 1965; Kakareko, 2002; Kornijów et al., 2005; Kornijów et al., 2016). It is however difficult to find an unequivocal confirmation of this fact in the fatty acid signatures of these fishes. It is due to the fact that these insects are a scarce source of DHA, but rich in C18 PUFA, whereas LA can serve as a precursor for ARA, while ALA can serve as a precursor for EPA and DHA (Bell and Tocher, 2009; Castro et al., 2016; Monroig et al., 2018). Therefore, consistent with previous controlled dietary studies in species that have the enzymatic capacity to synthesize HUFA from their precursors, which is highly likely in fish of the Cyprinidae family, their fatty acid composition may not directly reflect the diet (Garrido et al., 2020; Happel et al., 2016b; Janaranjani and Shu-Chien, 2020; Prigge et al., 2012). The absence of DHA in Chironomidae, like in Oligochaeta, might have induced a high conversion of dietary ALA to ensure sufficient DHA supply in roach and bream. It therefore cannot be excluded that the process of endogenous synthesis of HUFA in the organisms of individual studied fish species affects their fatty acid

composition, especially when experiencing food resources that are relatively poor in HUFA.

Samples representing eel from the Vistula Lagoon formed a relatively compact group pointing to low variability within the group of tested individuals (Figure 1). It is worth emphasising, however, that individuals covered by the study were characterised by a relatively narrow range of body length. Fatty acid signatures in eels strongly differed from fatty acid signatures determined for the remaining species. The major difference was the low proportion of EPA, DHA and ARA (less than 5% for each fatty acid) and the extremely high proportion of 18:1n-9 in European eel in comparison with other species. Another important feature was higher than in other species content of 14:0. Fatty acid signatures observed for eel from the Vistula Lagoon were the same as in the study by Tverin et al. (2019). The study of grey seal foraging habits included 11 fish species representing pelagic, demersal and coastal habitats of the Baltic Sea (Tverin et al., 2019). Like in our study, European eel was characterised by high content of 14:0 and MUFA, especially 16:1 and 18:1n-9, and low content of DHA. Stomach content and stable isotope analyses suggest that the European eel is an opportunistic carnivore, and adapts its diet to food availability (Bouchereau et al., 2009; Dörner et al., 2009). Based on the measured fatty acid signatures, it is difficult to conclude the type of prey consumed by eels from the Vistula Lagoon. The difficulty in correlating the fatty acid composition of European eel muscle tissue with suitable food resources was previously reported by Prigge et al. (2012). According to experimental studies, the authors found that fatty acid composition in eel muscle tissue seemed to be rather insensitive to fatty acids supplied in the diet. This phenomenon may be related to the biology of this species. The European eel, a catadromous species, lives in rivers, lakes, and estuaries, where it feeds and grows as a “yellow” eel, and after reaching the threshold size and physiological condition, including sufficient lipid reserves, it migrates back to its spawning site as a “silver” eel, and at this stage stops feeding. During the spawning migration, the energy required to travel thousands of kilometres and successfully reproduce is taken from the lipids accumulated in its body (Clevestam et al., 2011). This may explain the high content of MUFA in eels, as these fatty acids are heavily catabolised for energy in fish (Tocher, 2003). Moreover, it has been evidenced that during the maturation process, EPA and DHA are selectively moved from the muscle, and incorporated into gonads (Baeza et al., 2015; Nowosad et al., 2015). The composition of fatty acids in eels from the Vistula Lagoon may therefore indicate that these fish are already undergoing certain physiological and biochemical changes connected with the preparation of their organisms for reproductive migration. Note, it has been established that anguillid eels possess enzymatic capacities which allow modifying PUFA content in their tissues. Kissil et al. (1987) showed that European glass eel had the ability to convert LA into ARA. The complete enzymatic repertoire required for the biosynthesis of HUFA from C18 PUFA has been in turn confirmed for Japanese eel (*Anguilla japonica*) (Wang et al., 2014; Xu et al., 2020). This makes interpretations more difficult, because it may complicate any relationship between the contents of these fatty acids in the prey and tissues of consumers.

5. Conclusions

This study is the first step toward the application of fatty acids to highlight the role fish played in the food webs of the shallow coastal waters of the Baltic Sea. The presented results not only allowed for differentiating the studied fish species based on fatty acid signatures but also pointed to intraspecific changes in their diet. They expanded knowledge obtained based on stomach content analysis. However, our study revealed that further research, also experimental, is needed, in the case of many fish species, to take full advantage of the possibilities offered by fatty acids. The study conducted emphasised the importance of understanding the roles of different fatty acids in the organism's physiology and lipid metabolism before attempting to infer diet from fatty acid data. Particularly, the explanation of endogenous PUFA synthesis ability in different species of invertebrates and fish can considerably improve the usefulness of fatty acids in research on food webs in shallow coastal waters.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

- Airoidi, L., Beck, M.W., 2007. Loss, status and trends for coastal marine habitats of Europe. *Oceanogr. Mar. Biol.* 45, 345–405. <https://doi.org/10.1201/9781420050943.ch7>
- Amundsen, P.A., Sanchez-Hernandez, J., 2019. Feeding studies take guts - critical review and recommendations of methods for stomach contents analysis in fish. *J. Fish Biol.* 95, 1364–1373. <https://doi.org/10.1111/jfb.14151>
- Arrington, D.A., Winemiller, K.O., Loftus, W.F., Akin, S., 2002. How often do fishes "run on empty"? *Ecology* 83, 2145–2151. <https://doi.org/10.2307/3072046>
- Baeza, R., Mazzeo, I., Vilchez, M.C., Gallego, V., Penaranda, D.S., Perez, L., Asturiano, J.F., 2015. Relationship between sperm quality parameters and the fatty acid composition of the muscle, liver and testis of European eel. *Comp. Biochem. Phys. A* 181, 79–86. <https://doi.org/10.1016/j.cbpa.2014.11.022>
- Baker, R., Buckland, A., Sheaves, M., 2014. Fish gut content analysis: robust measures of diet composition. *Fish Fish.* 15, 170–177. <https://doi.org/10.1111/faf.12026>
- Bell, M.V., Batty, R.S., Dick, J.R., Fretwell, K., Navarro, J.C., Sargent, J.R., 1995. Dietary deficiency of docosahexaenoic acid impairs vision at low light intensities in juvenile herring (*Clupea harengus* L.). *Lipids* 30, 443–449. <https://doi.org/10.1007/bf02536303>
- Bell, M.V., Tocher, D.R., 2009. Biosynthesis of polyunsaturated fatty acids in aquatic ecosystems: general pathways and new directions. In: Kainz, M., Brett, M., Arts, M. (Eds.), *Lipids in Aquatic Ecosystems*. Springer, New York, 211–236. https://doi.org/10.1007/978-0-387-89366-2_9
- Bouchereau, J.L., Marques, C., Pereira, P., Guelorget, O., Vergne, Y., 2009. Food of the European eel *Anguilla anguilla* in the Mauguio lagoon (Mediterranean, France). *Acta Adriat.* 50, 159–170.
- Bouguenec, V., Giani, N., 1989. Aquatic oligochaetes as prey of invertebrates and vertebrates: a review. *Acta Oecol. Oecol. Appl.* 10, 177–196.
- Budge, S.M., Iverson, S.J., Koopman, H.N., 2006. Studying trophic ecology in marine ecosystems using fatty acids: A primer on analysis and interpretation. *Mar. Mammal Sci.* 22, 759–801. <https://doi.org/10.1111/j.1748-7692.2006.00079.x>
- Budge, S.M., Parrish, C.C., McKenzie, C.H., 2001. Fatty acid composition of phytoplankton, settling particulate matter and sediments at a sheltered bivalve aquaculture site. *Mar. Chem.* 76, 285–303. [https://doi.org/10.1016/s0304-4203\(01\)00068-8](https://doi.org/10.1016/s0304-4203(01)00068-8)
- Buckland, A., Baker, R., Loneragan, N., Sheaves, M., 2017. Standardising fish stomach content analysis: the importance of prey condition. *Fish. Res.* 196, 126–140. <https://doi.org/10.1016/j.fishres.2017.08.003>
- Casini, M., Hjelm, J., Molinero, J.C., Lovgren, J., Cardinale, M., Bartolino, V., Belgrano, A., Kornilovs, G., 2009. Trophic cascades promote threshold-like shifts in pelagic marine ecosystems. *Proc. Natl. Acad. Sci. U. S. A.* 106, 197–202. <https://doi.org/10.1073/pnas.0806649105>
- Castro, L.F.C., Tocher, D.R., Monroig, O., 2016. Long-chain polyunsaturated fatty acid biosynthesis in chordates: Insights into the evolution of Fads and Elovl gene repertoire. *Prog. Lipid Res.* 62, 25–40. <https://doi.org/10.1016/j.plipres.2016.01.001>
- Chaguaceda, F., Eklov, P., Scharnweber, K., 2020. Regulation of fatty acid composition related to ontogenetic changes and niche differentiation of a common aquatic consumer. *Oecologia* 193, 325–336. <https://doi.org/10.1007/s00442-020-04668-y>
- Clevestam, P.D., Ogonowski, M., Sjoberg, N.B., Wickstrom, H., 2011. Too short to spawn? Implications of small body size and swimming distance on successful migration and maturation of the European eel *Anguilla anguilla*. *J. Fish Biol.* 78, 1073–1089. <https://doi.org/10.1111/j.1095-8649.2011.02920.x>
- Collie, J.S., Wood, A.D., Jeffries, H.P., 2008. Long-term shifts in the species composition of a coastal fish community. *Can. J. Fish. Aquat. Sci.* 65, 1352–1365. <https://doi.org/10.1139/f08-048>
- Czesny, S.J., Rinchar, J., Hanson, S.D., Dettmers, J.M., Dabrowski, K., 2011. Fatty acid signatures of Lake Michigan prey fish and invertebrates: among-species differences and spatiotemporal variability. *Can. J. Fish. Aquat. Sci.* 68, 1211–1230. <https://doi.org/10.1139/f2011-048>
- Dalsgaard, J., St John, M., Kattner, G., Muller-Navarra, D., Hagen, W., 2003. Fatty acid trophic markers in the pelagic marine environment. *Adv. Mar. Biol.* 46, 225–340. [https://doi.org/10.1016/S0065-2881\(03\)46005-7](https://doi.org/10.1016/S0065-2881(03)46005-7)
- Demchuk, A.S., Uspenskiy, A.A., Golubkov, S.M., 2021. Abundance and feeding of fish in the coastal zone of the Neva Estuary, eastern Gulf of Finland. *Boreal Environ. Res.* 26, 1–16.
- Dmitrieva, O.A., Semenova, A.S., 2012. Seasonal dynamics and trophic interactions of phytoplankton and zooplankton in the Vistula Lagoon of the Baltic Sea. *Oceanology* 52, 785–789. <https://doi.org/10.1134/S0001437012060033>
- Dörner, H., Skov, C., Berg, S., Schulze, T., Beare, D.J., Van der Velde, G., 2009. Piscivory and trophic position of *Anguilla anguilla* in two lakes: importance of macrozoobenthos density. *J. Fish Biol.* 74, 2115–2131. <https://doi.org/10.1111/j.1095-8649.2009.02289.x>
- dos Santos, J., Jobling, M., 1991. Gastric emptying in cod, *Gadus morhua* L.: emptying and retention of indigestible solids. *J. Fish Biol.* 38, 187–197. <https://doi.org/10.1111/j.1095-8649.1991.tb03105.x>

- Eldson, T.S., 2010. Unraveling diet and feeding histories of fish using fatty acids as natural tracers. *J. Exp. Mar. Biol. Ecol.* 386, 61–68. <https://doi.org/10.1016/j.jembe.2010.02.004>
- Ezhova, E., Żmudziński, L., Maciejewska, K., 2005. Long-term trends in the macrozoobenthos of the Vistula Lagoon, southeastern Baltic Sea. Species composition and biomass distribution. *Bull. Sea Fish. Inst.* 1, 55–73.
- Filuk, J., Żmudziński, L., 1965. Feeding of the Vistula Lagoon ichthyofauna. *Rep. Sea Fish. Inst.* 13 A, 43–55 (in Polish).
- Folch, J., Lees, M., Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* 226, 497–509. [https://doi.org/10.1016/S0021-9258\(18\)64849-5](https://doi.org/10.1016/S0021-9258(18)64849-5)
- Futia, M.H., Colborne, S.F., Fisk, A.T., Gorsky, D., Johnson, T.B., Lantry, B.F., Lantry, J.R., Rinchar, J., 2021. Comparisons among three diet analyses demonstrate multiple patterns in the estimated adult diet of a freshwater piscivore, *Salvelinus namaycush*. *Ecol. Indic.* 127. <https://doi.org/10.1016/j.ecolind.2021.107728>
- Galloway, A.W.E., Budge, S.M., 2020. The critical importance of experimentation in biomarker-based trophic ecology. *Philos. Trans. R. Soc. B: Biol. Sci.* 375, 20190638. <https://doi.org/10.1098/rstb.2019.0638>
- Galloway, A.W.E., Winder, M., 2015. Partitioning the relative importance of phylogeny and environmental conditions on phytoplankton fatty acids. *Plos One* 10. <https://doi.org/10.1371/journal.pone.0130053>
- Garrido, D., Monroig, O., Galindo, A., Betancor, M.B., Perez, J.A., Kabeya, N., Marrero, M., Rodríguez, C., 2020. Lipid metabolism in *Tinca tinca* and its n-3 LC-PUFA biosynthesis capacity. *Aquaculture* 523, 735147. <https://doi.org/10.1016/j.aquaculture.2020.735147>
- Gladyshev, M.I., Sushchik, N.N., Anishchenko, O.V., Makhutova, O.N., Kolmakov, V.I., Kalachova, G.S., Kolmakova, A.A., Dubovskaya, O.P., 2011. Efficiency of transfer of essential polyunsaturated fatty acids versus organic carbon from producers to consumers in a eutrophic reservoir. *Oecologia* 165, 521–531. <https://doi.org/10.1007/s00442-010-1843-6>
- Gladyshev, M.I., Sushchik, N.N., Tolomeev, A.P., Dgebuadze, Y.Y., 2018. Meta-analysis of factors associated with omega-3 fatty acid contents of wild fish. *Rev. Fish Biol. Fish.* 28, 277–299. <https://doi.org/10.1007/s11160-017-9511-0>
- Glazunova, A.A., Polunina, J.J., 2013. Peculiarities of under-ice zooplankton in the Curonian and Vistula Lagoons of the Baltic Sea. *Inland Water Biol.* 6, 301–304. <https://doi.org/10.1134/S1995082913040081>
- Goedkoop, W., Sonesten, L., Ahlgren, G., Boberg, M., 2000. Fatty acids in profundal benthic invertebrates and their major food resources in Lake Erken, Sweden: seasonal variation and trophic indications. *Can. J. Fish. Aquat. Sci.* 57, 2267–2279. <https://doi.org/10.1139/f00-201>
- Golubkov, S., Tiunov, A., Golubkov, M., 2021. Food-web modification in the eastern Gulf of Finland after invasion of *Marenzelleria arctica* (Spionidae, Polychaeta). *Neobiota* 66, 75–94. <https://doi.org/10.3897/neobiota.66.63847>
- Guo, F., Bunn, S.E., Brett, M.T., Kainz, M.J., 2017. Polyunsaturated fatty acids in stream food webs - high dissimilarity among producers and consumers. *Freshw. Biol.* 62, 1325–1334. <https://doi.org/10.1111/fwb.12956>
- Hansson, S., Arrhenius, F., Nellbring, S., 1997. Diet and growth of pikeperch (*Stizostedion lucioperca* L.) in a Baltic Sea area. *Fish. Res.* 31, 163–167. [https://doi.org/10.1016/S0165-7836\(97\)00022-2](https://doi.org/10.1016/S0165-7836(97)00022-2)
- Happel, A., Creque, S., Rinchar, J., Hooeek, T., Bootsma, H., Janssen, J., Jude, D., Czesny, S., 2015. Exploring yellow perch diets in Lake Michigan through stomach content, fatty acids, and stable isotope ratios. *J. Great Lakes Res.* 41, 172–178. <https://doi.org/10.1016/j.jglr.2015.03.025>
- Happel, A., Stratton, L., Kolb, C., Hays, C., Rinchar, J., Czesny, S., 2016a. Evaluating quantitative fatty acid signature analysis (QFASA) in fish using controlled feeding experiments. *Can. J. Fish. Aquat. Sci.* 73, 1222–1229. <https://doi.org/10.1139/cjfas-2015-0328>
- Happel, A., Stratton, L., Patridge, R., Rinchar, J., Czesny, S., 2016b. Fatty-acid profiles of juvenile lake trout reflect experimental diets consisting of natural prey. *Freshw. Biol.* 61, 1466–1476. <https://doi.org/10.1111/fwb.12786>
- Henrotte, E., Kpogue, D., Mandiki, S.N.M., Wang, N., Douxfils, J., Dick, J., Tocher, D., Kestemond, P., 2011. n-3 and n-6 fatty acid bioconversion abilities in Eurasian perch (*Perca fluviatilis*) at two developmental stages. *Aquacult. Nutr.* 17, 216–225. <https://doi.org/10.1111/j.1365-2095.2010.00754.x>
- Hjelm, J., Persson, L., Christensen, B., 2000. Growth, morphological variation and ontogenetic niche shifts in perch (*Perca fluviatilis*) in relation to resource availability. *Oecologia* 122, 190–199. <https://doi.org/10.1007/pl00008846>
- Hyslop, E.J., 1980. Stomach contents analysis – a review of methods and their application. *J. Fish Biol.* 17, 411–429. <https://doi.org/10.1111/j.1095-8649.1980.tb02775.x>
- Ishizaki, Y., Masuda, R., Uematsu, K., Shimizu, K., Arimoto, M., Takeuchi, T., 2001. The effect of dietary docosahexaenoic acid on schooling behaviour and brain development in larval yellowtail. *J. Fish Biol.* 58, 1691–1703. <https://doi.org/10.1006/jfbi.2001.1579>
- Iverson, S.J., Field, C., Bowen, W.D., Blanchard, W., 2004. Quantitative fatty acid signature analysis: a new method of estimating predator diets. *Ecol. Monogr.* 74, 211–235. <https://doi.org/10.1890/02-4105>
- Iverson, S.J., Springer, A.M., Kitaysky, A.S., 2007. Seabirds as indicators of food web structure and ecosystem variability: qualitative and quantitative diet analyses using fatty acids. *Mar. Ecol. Prog. Ser.* 352, 235–244. <https://doi.org/10.3354/meps07073>
- Janaranjani, M., Shu-Chien, A.C., 2020. Complete repertoire of long-chain polyunsaturated fatty acids biosynthesis enzymes in a cyprinid, silver barb (*Barbonymus gonionotus*): cloning, functional characterization and dietary regulation of Elovl2 and Elovl4. *Aquacult. Nutr.* 26, 1835–1853. <https://doi.org/10.1111/anu.13133>
- Janer, G., Navarro, J.C., Porte, C., 2007. Exposure to TBT increases accumulation of lipids and alters fatty acid homeostasis in the ramshorn snail *Marisa cornuarietis*. *Comp. Biochem. Phys. C* 146, 368–374. <https://doi.org/10.1016/j.cbpc.2007.04.009>
- Kabeya, N., Fonseca, M.M., Ferrier, D.E.K., Navarro, J.C., Bay, L.K., Francis, D.S., Tocher, D., Castro, L.F.C., Monroig, O., 2018. Genes for de novo biosynthesis of omega-3 polyunsaturated fatty acids are widespread in animals. *Sci. Adv.* 4, eaar6849. <https://doi.org/10.1126/sciadv.aar6849>
- Kakareko, T., 2002. The importance of benthic fauna in the diet of small common bream *Abramis brama* [L.], roach *Rutilus rutilus* [L.], pikeperch *Sander lucioperca* [L.] and ruffe *Gymnocephalus cernuus* [L.] in the Włocławek Reservoir. *Arch. Pol. Fish.* 10, 221–231.
- Käkelä, R., Käkelä, A., Kahle, S., Becker, P.H., Kelly, A., Furness, R.W., 2005. Fatty acid signatures in plasma of captive herring gulls as indicators of demersal or pelagic fish diet. *Mar. Ecol. Prog. Ser.* 293, 191–200. <https://doi.org/10.3354/meps293191>
- Keinanen, M., Kakela, R., Ritvanen, T., Myllylä, T., Ponni, J., Vuorinen, P.J., 2017. Fatty acid composition of sprat (*Sprattus sprattus*) and herring (*Clupea harengus*) in the Baltic Sea as potential prey for salmon (*Salmo salar*). *Helgol. Mar. Res.* 71, 4. <https://doi.org/10.1186/s10152-017-0484-0>
- Kelly, J.R., Scheibling, R.E., 2012. Fatty acids as dietary tracers in benthic food webs. *Mar. Ecol. Prog. Ser.* 446, 1–22. <https://doi.org/10.3354/meps09559>
- Kirsch, P.E., Iverson, S.J., Bowen, W.D., Kerr, S.R., Ackman, R.G., 1998. Dietary effects on the fatty acid signature of whole At-

- lantic cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci. 55, 1378–1386. <https://doi.org/10.1139/cjfas-55-6-1378>
- Kissil, G.W., Youngson, A., Cowey, C.B., 1987. Capacity of the European eel (*Anguilla anguilla*) to elongate and desaturate dietary linoleic acid. J. Nutr. 117, 1379–1384. <https://doi.org/10.1093/jn/117.8.1379>
- Kornijów, R., 2018. Ecosystem of the Polish part of the Vistula Lagoon from the perspective of alternative stable states concept, with implications for management issues. Oceanologia 60 (3), 390–404. <https://doi.org/10.1016/j.oceano.2018.02.004>
- Kornijów, R., Karpowicz, M., Ejsmont-Karabin, J., Nawrocka, L., de Eyto, E., Grzonkowski, K., Magnuszewski, A., Jakubowska, A., Wodzinowski, T., Woźniczka, A., 2020. Patchy distribution of phyto- and zooplankton in large and shallow lagoon under ice cover and resulting trophic interactions. Mar. Freshw. Res. 71, 1327–1341. <https://doi.org/10.1071/Mf19259>
- Kornijów, R., Measey, G.J., Moss, B., 2016. The structure of the littoral: effects of waterlily density and perch predation on sediment and plant-associated macroinvertebrate communities. Freshw. Biol. 61, 32–50. <https://doi.org/10.1111/fwb.12674>
- Kornijów, R., Pawlikowski, K., Błędzki, L.A., Drgas, A., Piwosz, K., Ameryk, A., Calkiewicz, J., 2021. Co-occurrence and potential resource partitioning between oligochaetes and chironomid larvae in a sediment depth gradient. Aquat. Sci. 83, 51. <https://doi.org/10.1007/s00027-021-00800-z>
- Kornijów, R., Vakkilainen, K., Horppila, J., Luokkanen, E., Kairesalo, T., 2005. Impacts of a submerged plant (*Elodea canadensis*) on interactions between roach (*Rutilus rutilus*) and its invertebrate prey communities in a lake littoral zone. Freshw. Biol. 50, 262–276. <https://doi.org/10.1111/j.1365-2427.2004.01318.x>
- Koussoroplis, A.-M., Bec, A., Perga, M.-E., Koutrakis, E., Bourdier, G., Desvillettes, C., 2011. Fatty acid transfer in the food web of a coastal Mediterranean lagoon: Evidence for high arachidonic acid retention in fish. Estuar. Coast. Shelf S. 91, 450–461. <https://doi.org/10.1016/j.ecss.2010.11.010>
- Kownacka, J., Calkiewicz, J., Kornijów, R., 2020. A turning point in the development of phytoplankton in the Vistula Lagoon (southern Baltic Sea) at the beginning of the 21st century. Oceanologia 62 (4), 538–555. <https://doi.org/10.1016/j.oceano.2020.08.004>
- Kraufvelin, P., Pekcan-Hekim, Z., Bergstrom, U., Florin, A.-B., Lehikoinen, A., Mattila, J., et al., 2018. Essential coastal habitats for fish in the Baltic Sea. Estuar. Coast. Shelf S. 204, 14–30. <https://doi.org/10.1016/j.ecss.2018.02.014>
- Lammens, E.H.R.R., Geursen, J., McGillivray, P.J., 1985. Diet shifts, feeding efficiency and coexistence of bream *Abramis brama*, roach *Rutilus rutilus* and white bream *Blicca bjoerkna* in hypertrophic lakes. In: Kullander, S.O., Fernholm, B. (Eds.), Proceedings of the Fifth Congress of European Ichthyologists. Department of Vertebrate Zoology, Swedish Museum of Natural History, Stockholm, 153–162.
- Lammens, E.H.R.R., Hoogenboezem, W., 1991. Diets and feeding behaviour. In: Winfield, I.J., Nelson, J.S. (Eds.), Cyprinid fishes: systematics, biology and exploitation. Chapman and Hall, London, 353–376. https://doi.org/10.1007/978-94-011-3092-9_12
- Lau, D.C.P., Vrede, T., Pickova, J., Goedkoop, W., 2012. Fatty acid composition of consumers in boreal lakes - variation across species, space and time. Freshw. Biol. 57, 24–38. <https://doi.org/10.1111/j.1365-2427.2011.02690.x>
- Lee, R.F., Hagen, W., Kattner, G., 2006. Lipid storage in marine zooplankton. Mar. Ecol. Prog. Ser. 307, 273–306. <https://doi.org/10.3354/meps307273>
- Legeżyńska, J., Kędra, M., Walkusz, W., 2014. Identifying trophic relationships within the high Arctic benthic community: how much can fatty acids tell? Mar. Biol. 161, 821–836. <https://doi.org/10.1007/s00227-013-2380-8>
- Lehtonen, H., Hansson, S., Winkler, H., 1996. Biology and exploitation of pikeperch, *Stizostedion lucioperca* (L), in the Baltic Sea area. Ann. Zool. Fenn. 33, 525–535.
- Lind, Y., Huovila, T., Kakela, R., 2018. A retrospective study of fatty acid composition in Baltic herring (*Clupea harengus membras*) caught at three locations in the Baltic Sea (1973-2009). ICES J. Mar. Sci. 75, 330–339. <https://doi.org/10.1093/icesjms/fsx127>
- Maazouzi, C., Medoc, V., Pihan, J.-C., Masson, G., 2011. Size-related dietary changes observed in young-of-the-year pumpkinseed (*Lepomis gibbosus*): stomach contents and fatty acid analyses. Aquat. Ecol. 45, 23–33. <https://doi.org/10.1007/s10452-010-9320-1>
- Makhotova, O.N., Stoyanov, K.N., 2021. Fatty acid content and composition in tissues of Baikal grayling (*Thymallus baicalensis*), with a special focus on DHA synthesis. Aquac. Int. 29, 2415–2433. <https://doi.org/10.1007/s10499-021-00755-w>
- Makhotova, O.N., Sushchik, N.N., Gladyshev, M.I., Ageev, A.V., Pryanichnikova, E.G., Kalachova, G.S., 2011. Is the fatty acid composition of freshwater zoobenthic invertebrates controlled by phylogenetic or trophic factors? Lipids 46, 709–721. <https://doi.org/10.1007/s11745-011-3566-9>
- McLusky, D.S., Elliott, M., 2004. The Estuarine Ecosystem; Ecology, Threats and Management. Oxford University Press, Oxford, 214 pp.
- Merad, I., Bellenger, S., Hichami, A., Khan, N.A., Soltani, N., 2018. Effect of cadmium exposure on essential omega-3 fatty acids in the edible bivalve *Donax trunculus*. Environ. Sci. Pollut. R. 25, 18242–18250. <https://doi.org/10.1007/s11356-017-9031-4>
- Möllmann, C., Diekmann, R., Müller-Karulis, B., Kornilovs, G., Plikshs, M., Axe, P., 2009. Reorganization of a large marine ecosystem due to atmospheric and anthropogenic pressure: a discontinuous regime shift in the Central Baltic Sea. Glob. Change Biol. 15, 1377–1393. <https://doi.org/10.1111/j.1365-2486.2008.01814.x>
- Möllmann, C., Müller-Karulis, B., Kornilovs, G., St John, M.A., 2008. Effects of climate and overfishing on zooplankton dynamics and ecosystem structure: regime shifts, trophic cascade, and feedback loops in a simple ecosystem. ICES J. Mar. Sci. 65, 302–310. <https://doi.org/10.1093/icesjms/fsm197>
- Monroig, O., Kabeya, N., 2018. Desaturases and elongases involved in polyunsaturated fatty acid biosynthesis in aquatic invertebrates: a comprehensive review. Fish. Sci. 84, 911–928. <https://doi.org/10.1007/s12562-018-1254-x>
- Monroig, O., Tocher, D.R., Castro, L.F.C., 2018. Polyunsaturated fatty acid biosynthesis and metabolism in fish. In: Burdge, G.C. (Ed.), Polyunsaturated fatty acid metabolism. AOCs Press, London, 31–60. <https://doi.org/10.1016/B978-0-12-811230-4.00003-X>
- Mourente, G., Tocher, D.R., Sargent, J.R., 1991. Specific accumulation of docosahexaenoic acid (22:6n-3) in brain lipids during development of juvenile turbot *Scophthalmus maximus* L. Lipids 26, 871–877. <https://doi.org/10.1007/bf02535970>
- Mustamaki, N., Cederberg, T., Mattila, J., 2014. Diet, stable isotopes and morphology of Eurasian perch (*Perca fluviatilis*) in littoral and pelagic habitats in the northern Baltic Proper. Environ. Biol. Fish. 97, 675–689. <https://doi.org/10.1007/s10641-013-0169-8>
- Nagelkerke, L.A.J., Sibbing, F.A., 1996. Efficiency of feeding on zebra mussel (*Dreissena polymorpha*) by common bream (*Abramis brama*), white bream (*Blicca bjoerkna*), and roach (*Rutilus rutilus*): The effects of morphology and behavior. Can. J. Fish. Aquat. Sci. 53, 2847–2861. <https://doi.org/10.1139/cjfas-53-12-2847>
- Nawrocka, L., Kobos, J., 2011. The trophic state of the Vistula Lagoon: an assessment based on selected biotic and abiotic parameters according to the Water Framework Directive. Oceanologia

- 53 (3), 881–894. <https://doi.org/10.5697/oc.53-3.881>
- Nielsen, J.M., Clare, E.L., Hayden, B., Brett, M.T., Kratina, P., 2018. Diet tracing in ecology: Method comparison and selection. *Methods Ecol. Evol.* 9, 278–291. <https://doi.org/10.1111/2041-210x.12869>
- Nowosad, J., Kucharczyk, D., Łuczyńska, J., Targońska, K., Czarkowski, T.K., Biłas, M., Krejszef, S., Horváth, L., Müller, T., 2015. Changes in European eel ovary development and body and ovary chemistry during stimulated maturation under controlled conditions: preliminary data. *Aquacult. Int.* 23, 13–27. <https://doi.org/10.1007/s10499-014-9794-2>
- Ojaveer, H., Lankov, A., Raid, T., Pllumae, A., Klais, R., 2018. Selecting for three copepods-feeding of sprat and herring in the Baltic Sea. *ICES J. Mar. Sci.* 75, 2439–2449. <https://doi.org/10.1093/icesjms/fsx249>
- Parrish, C.C., 2009. Essential fatty acids in aquatic food webs. In: Arts, M.T., Brett, M.T., Kainz, M.J. (Eds.), *Lipids in Aquatic Ecosystems*. Springer, New York, 309–326. https://doi.org/10.1007/978-0-387-89366-2_13
- Paturej, E., Gutkowska, A., 2015. The effect of salinity levels on the structure of zooplankton communities. *Arch. Biol. Sci.* 67, 483–492. <https://doi.org/10.2298/abs140910012p>
- Paturej, E., Gutkowska, A., Koszalka, J., Bowszys, M., 2017. Effect of physicochemical parameters on zooplankton in the brackish, coastal Vistula Lagoon. *Oceanologia* 59 (1), 49–56. <https://doi.org/10.1016/j.oceano.2016.08.001>
- Pawlikowski, K., Kornijów, R., 2019. Role of macrophytes in structuring littoral habitats in the Vistula Lagoon (southern Baltic Sea). *Oceanologia* 61 (1), 26–37. <https://doi.org/10.1016/j.oceano.2018.05.003>
- Persson, A., Brönmark, C., 2002. Foraging capacities and effects of competitive release on ontogenetic diet shift in bream, *Abramis brama*. *Oikos* 97, 271–281. <https://doi.org/10.1034/j.1600-0706.2002.970213.x>
- Persson, J., Vrede, T., 2006. Polyunsaturated fatty acids in zooplankton: variation due to taxonomy and trophic position. *Freshw. Biol.* 51, 887–900. <https://doi.org/10.1111/j.1365-2427.2006.01540.x>
- Prejs, A., Lewandowski, K., Stańczykowska-Piotrowska, A., 1990. Size-selective predation by roach (*Rutilus rutilus*) on zebra mussel (*Dreissena polymorpha*): field studies. *Oecologia* 83, 378–384. <https://doi.org/10.1007/bf00317563>
- Prigge, E., Malzahn, A.M., Zumholz, K., Hanel, R., 2012. Dietary effects on fatty acid composition in muscle tissue of juvenile European eel, *Anguilla anguilla* (L.). *Helgol. Mar. Res.* 66, 51–61. <https://doi.org/10.1007/s10152-011-0246-3>
- Psuty, I., Wilkońska, H., 2009. The stability of fish assemblages under unstable conditions: a ten-year series from the Polish part of the Vistula Lagoon. *Arch. Pol. Fish.* 17, 65–76. <https://doi.org/10.2478/v10086-009-0004-1>
- Sawyer, J.M., Arts, M.T., Arhonditsis, G., Diamond, M.L., 2016. A general model of polyunsaturated fatty acid (PUFA) uptake, loss and transformation in freshwater fish. *Ecol. Model.* 323, 96–105. <https://doi.org/10.1016/j.ecolmodel.2015.12.004>
- Scharnweber, K., Chaguaceda, F., Eklov, P., 2021. Fatty acid accumulation in feeding types of a natural freshwater fish population. *Oecologia* 196, 53–63. <https://doi.org/10.1007/s00442-021-04913-y>
- Specziár, A., Rezsű, E.T., 2009. Feeding guilds and food resource partitioning in a lake fish assemblage: an ontogenetic approach. *J. Fish Biol.* 75, 247–267. <https://doi.org/10.1111/j.1095-8649.2009.02283.x>
- Specziár, A., Tölg, L., Biró, P., 1997. Feeding strategy and growth of cyprinids in the littoral zone of Lake Balaton. *J. Fish Biol.* 51, 1109–1124. <https://doi.org/10.1111/j.1095-8649.1997.tb01130.x>
- StatSoft, 2011. *Electronic statistics textbook* StatSoft. Tulsa, OK, USA.
- Stolarski, J., 1995. Sichel (*Pelecus cultratus*, L.) from the Vistula Lagoon. *Bull. Sea Fish. Inst.* 2, 11–21.
- Stowasser, G., Pond, D.W., Collins, M.A., 2009. Using fatty acid analysis to elucidate the feeding habits of Southern Ocean mesopelagic fish. *Mar. Biol.* 156, 2289–2302. <https://doi.org/10.1007/s00227-009-1256-4>
- Strandberg, U., Hiltunen, M., Jelkänen, E., Taipale, S.J., Kainz, M.J., Brett, M.T., Kankaala, P., 2015. Selective transfer of polyunsaturated fatty acids from phytoplankton to planktivorous fish in large boreal lakes. *Sci. Tot. Environ.* 536, 858–865. <https://doi.org/10.1016/j.scitotenv.2015.07.010>
- Sushchik, N.N., Gladyshev, M.I., Kalachova, G.S., Makhutova, O.N., Ageev, A.V., 2006. Comparison of seasonal dynamics of the essential PUFA contents in benthic invertebrates and grayling *Thymallus arcticus* in the Yenisei river. *Comp. Biochem. Phys. B* 145, 278–287. <https://doi.org/10.1016/j.cbpb.2006.05.014>
- Sushchik, N.N., Rudchenko, A.E., Gladyshev, M.I., 2017. Effect of season and trophic level on fatty acid composition and content of four commercial fish species from Krasnoyarsk Reservoir (Siberia, Russia). *Fish. Res.* 187, 178–187. <https://doi.org/10.1016/j.fishres.2016.11.016>
- Sutton, T.M., Cyterski, M.J., Ney, J.J., Duval, M.C., 2004. Determination of factors influencing stomach content retention by striped bass captured using gillnets. *J. Fish Biol.* 64, 903–910. <https://doi.org/10.1111/j.1095-8649.2004.0358.x>
- Szlinder-Richert, J., Usydus, Z., Wyszynski, M., Adamczyk, M., 2010. Variation in fat content and fatty-acid composition of the Baltic herring *Clupea harengus membras*. *J. Fish Biol.* 77, 585–599. <https://doi.org/10.1111/j.1095-8649.2010.02696.x>
- Thiemann, G.W., Iverson, S.J., Stirling, I., 2008. Polar bear diets and Arctic marine food webs: insights from fatty acid analysis. *Ecol. Monogr.* 78, 591–613. <https://doi.org/10.1890/07-1050.1>
- Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. *Rev. Fish. Sci.* 11, 107–184. <https://doi.org/10.1080/713610925>
- Tocher, D.R., 2010. Fatty acid requirements in ontogeny of marine and freshwater fish. *Aquac. Res.* 41, 717–732. <https://doi.org/10.1111/j.1365-2109.2008.02150.x>
- Trushenski, J.T., Rombenso, A.N., 2020. Trophic levels predict the nutritional essentiality of polyunsaturated fatty acids in fish-introduction to a special section and a brief synthesis. *N. Am. J. Aquacult.* 82, 241–250. <https://doi.org/10.1002/naaq.10137>
- Tverin, M., Esparza-Salas, R., Strömberg, A., Tang, P., Kokkonen, I., Herrero, A., Kauhala, K., Karlsson, O., Tiilikainen, R., Vetemaa, M., Sinisalo, T., Käkälä, R., Lundström, K., 2019. Complementary methods assessing short and long-term prey of a marine top predator - Application to the grey seal-fishery conflict in the Baltic Sea. *Plos One* 14, e0208694. <https://doi.org/10.1371/journal.pone.0208694>
- Usydus, Z., Szlinder-Richert, J., Adamczyk, M., Szatkowska, U., 2011. Marine and farmed fish in the Polish market: Comparison of the nutritional value. *Food Chem.* 126, 78–84. <https://doi.org/10.1016/j.foodchem.2010.10.080>
- Wang, S.Q., Monroig, O., Tang, G.X., Zhang, L., You, C.H., Tocher, D.R., Li, Y., 2014. Investigating long-chain polyunsaturated fatty acid biosynthesis in teleost fish: functional characterization of fatty acyl desaturase (Fads2) and Elovl5 elongase in the catadromous species, Japanese eel *Anguilla japonica*. *Aquaculture* 434, 57–65. <https://doi.org/10.1016/j.aquaculture.2014.07.016>
- Wasmund, N., Uhlig, S., 2003. Phytoplankton trends in the Baltic Sea. *ICES J. Mar. Sci.* 60, 177–186. [https://doi.org/10.1016/S1054-3139\(02\)00280-1](https://doi.org/10.1016/S1054-3139(02)00280-1)

- Wiśniewski, R.J., 1978. Effect of predators on Tubificidae groupings and their production in lakes. *Pol. J. Ecol.* 26, 493–512.
- Xu, W.J., Wang, S.Q., You, C.H., Zhang, Y.L., Monroig, O., Tocher, D.R., Li, Y, 2020. The catadromous teleost *Anguilla japonica* has a complete enzymatic repertoire for the biosynthesis of docosahexaenoic acid from alpha-linolenic acid: cloning and functional characterization of an Elovl2 elongase. *Comp. Biochem. Phys. B* 240, 110373. <https://doi.org/10.1016/j.cbpb.2019.110373>
- Żmudziński, L., 1996. The effect of the introduction of the American species *Marenzelleria viridis* (Polychaeta: Spionidae) on the benthic ecosystem of Vistula lagoon. *Mar. Ecol.* 17, 221–226. <https://doi.org/10.1111/j.1439-0485.1996.tb00503.x>