Trophic Ecology of the European Eel (*Anguilla anguilla*) across Different Salinity Habitats Inferred from Fatty Acid and Stable Isotope Analysis

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Abstract

We combined fatty acid (FA) and stable isotope (SI) analyses to investigate the trophic ecology of different stages of European eels (*Anguilla anguilla*) across sea (SW), brackish (BW), and freshwater (FW) habitats. Salinity was the main driver of differences in the biochemical composition, and the greatest variation occurred between SW and FW eels. SW eels had a higher content of the FA indicator of carnivory, as well as the highest stable isotope ratios (C, N). In contrast, FW eels exhibited the highest lipid content and omega-6 polyunsaturated FA, but the lowest stable isotope ratios, suggesting major dietary differences between the eels in these two habitats. While the biochemical composition of BW eels was closer to those of SW eels, BW eels had the largest SI range, indicating higher dietary plasticity. FW individuals had better overall condition compared to SW eels. Independent of habitat, larger individuals were in the best condition, and had higher lipid content and monounsaturated FA. These findings suggest a biological advantage for eels to maintain a catadromous life-history strategy.

**Keywords** $\delta^{13}$C, $\delta^{15}$N, habitat shifting, MUFA, PUFA
Introduction

The European eel (*Anguilla anguilla*) is a facultatively catadromous species (Tzeng et al. 2000; Daverat et al. 2006). Adult eels reproduce and spawn in the Sargasso Sea. From there, they drift along the Antilles Current, Gulf Stream, and North Atlantic Drift as leptocephalus larvae until they reach the African and European continental shelves, where they metamorphose into translucent “glass” eels (Tesch 1977; Tzeng et al. 2000). Glass eels then recruit to various marine/seawater (SW; e.g. open sea and coastal habitats), brackish water (BW; e.g. salt marshes, lagoons, estuaries), and/or freshwater (FW; e.g. rivers, lakes) habitats, where they spend several years to decades feeding and growing as “yellow” eels (Tesch 1977; Moriarty 2003). Although the details are not yet fully understood (e.g. Larsson et al. 1990; Svedäng and Wickström 1997), current consensus is that yellow eels go through their final transformation, i.e. “silvering”, once they accumulate sufficient energy stores. Silver eels become sexually mature while migrating back to the Sargasso Sea (Palstra and van den Thillart 2010).

During their continental residency, eels adopt different life history strategies. Some individuals spend the entire period in either SW or FW habitats, while others exhibit a habitat-shifting strategy, moving back and forth between SW and FW habitats. The reason(s) for this plasticity in life history strategy (i.e. traditionally recognized FW phase, SW residency, and habitat shifting) is/are unknown. Gross (1987) argued diadromy (i.e. anadromy and catadromy) is evolutionarily favored when it enhances growth and/or reproductive success (i.e. when it increases fitness). When examining the evolutionary origin of both catadromous and anadromous migrations, Gross et al. (1987, 1988) specifically suggested that food availability and differences in productivity between FW and SW systems may explain such habitat-shifting behavior. In other words, the greater the food intake, the higher the fitness gain due to increased body growth and reproductive success (Gross et al. 1988). In support of this hypothesis, Thibault et al. (2007) observed that American eels (*Anguilla rostrata*) residing in the
estuary of the St. Jean River (Québec, Canada) were characterized by higher growth rates than those captured from the less productive St. Jean River and adjacent Sorois Lake. Arai and Chino (2012) suggested that other factors, including competition within and among species, predation, habitat use, and the general abiotic conditions in each environment may drive the habitat-shifting behavior of anguillid eels.

In this context, the integrated use of fatty acid (FA) and stable isotope (SI) biomarkers may further clarify both the trophic ecology and movement patterns of A. anguilla, ultimately providing a better understanding of the ecological advantages of the different life history strategies. Biomarkers, such as individual FA, and FA and SI ratios, have been applied to study long-term diet and feeding habits (Iverson et al. 2004), habitat use and preference (Cucherousset et al. 2011), and migration patterns (Harrod et al. 2005; McCarthy and Waldron 2000) in aquatic organisms. Since the FA and/or SI profiles of consumers reflect that of their diets, they can be used as indirect markers of the habitat that was the source of the food consumed. FA are useful trophic biomarkers because most aquatic consumers cannot synthesize certain essential nutrients, including the long chain polyunsaturated FA (LC-PUFA) 20:5n-3 (eicosapentaenoic acid, EPA), 22:6n-3 (docosahexaenoic acid, DHA), and 20:4n-6 (arachidonic acid, ARA), in sufficient amounts to meet their physiological needs (Parrish 2009, 2013). Therefore, they must be acquired through diet (Parrish 2008). As FA are transferred in a relatively conservative manner (Iverson et al. 2004), it is possible to study consumer feeding habits (Iverson et al. 2004), and to infer habitat use (Prigge et al. 2012), by looking at the FA composition of consumer tissues. For instance, Prigge et al. (2012), found that eels obtained from a fish farm in Germany, and fed a FW diet composed of Chironomidae larvae, amphipods (Gammarus pulex), and common roach (Rutilus rutilus), maintained the same general patterns of low n-3/n-6 and EPA/ARA ratios as their prey items.

Stable nitrogen ($^{15}$N/$^{14}$N or $\delta^{15}$N) and carbon ($^{13}$C/$^{12}$C or $\delta^{13}$C) isotope ratios are the most common SI biomarkers used in trophic ecology and migration studies (Hobson 1999; Harrod et
In particular, $\delta^{15}N$ is generally used to study an organism’s trophic position due to its predictable $^{15}N$ stepwise increase of $2\% - 4\%$ (Minagawa and Wada 1984; Post 2002) between a consumer and its source. In contrast, the relative change in $^{13}C$ between consecutive trophic positions is negligible (i.e. < 1%; McConnaughey and McRoy 1979). Since primary producers have characteristic stable C isotope signatures (McConnaughey and McRoy 1979; Budge et al. 2008), $\delta^{13}C$ is a useful indicator of primary food sources and, indirectly, of feeding habitats. For instance, clear isotopic differences exist between FW and SW ecosystems due to increasing $\delta^{13}C$ isotope ratios of organic matter from FW to SW habitats (Hobson 1999; McCarthy and Waldron 2000). This gradient is caused by the variable contribution of C$_3$ vs C$_4$ plants in the two biomes (Peterson and Fry 1987). In addition, FW ecosystems typically have lower $\delta^{15}N$ values than their SW counterparts, due to the proportionally greater influence of terrestrial inputs on these systems (Owens 1988; McCarthy and Waldron 2000). As European eels may feed in multiple habitats (SW, BW, FW) during their life cycle, FA and SI analysis may hence further elucidate their feeding habits and preferences.

European eels, and anguillid eels in general, are opportunistic predators, and their diet typically includes benthic polychaetes, molluscs, crustaceans, insect larvae, and/or fishes (De Nie 1982; Bouchereau et al. 2009; Kaifu et al. 2012). However, their diet may vary across habitats, seasons, life stages, sizes (Sinha and Jones 1967; Bouchereau et al. 2009; Prigge et al. 2012), and head morphologies (Proman and Reynolds 2000; De Meyer et al. 2016). The diet of European eels from the upper zone of the Tagus Estuary (Portugal) shifted from amphipods and shore crabs (Carcinus maenas) to polychaetes, bivalves, and shrimps in more saline areas (Costa et al. 1992). Moreover, BW individuals of Japanese eels (Anguilla japonica), collected in Kojima Bay (Japan) were found to rely more on the pelagic food web, mainly feeding on mud shrimps (Upogebia major); whereas their FW counterparts, collected in the Asahi River, depended more on the littoral food web, feeding on the crayfish Procambarus clarkia (Kaifu et al. 2012). In addition, younger yellow European eels (< 35 cm total length) from Lake Manzalah would have a different diet from the larger eels.
(Egypt) mainly fed on crustaceans, insect larvae (from Family Chironomidae and Order Odonata), and molluscs, with only a small contribution of fish (Ezzat and El-Serrafy 1977). In contrast, fish represented the main food item of older eels measuring between 39.5 and 55.5 cm, and the only food item for those individuals with total lengths > 55.5 cm (Ezzat and El-Serrafy 1977). Similarly, FW European eels collected in Austevoll, Norway, were found to feed mainly upon insects (from Chironomidae, and Orders Thrichoptera and Ephemeroptera) and molluscs (from Classes Bivalvia and Gastropoda) (Sagen 1983). Fish were also included in the diet of larger individuals (> 50 cm): mainly three-spined stickleback (Gasterosteus aculeatus), but also glass eels in the spring, and salmon (Salmo salar) in the fall (Sagen 1983).

Cucherousset et al. (2011) observed that yellow eels with broader heads occupied higher trophic positions than those with narrower heads, resulting from a diet richer in fish and, in general, in larger and/or harder-body prey.

The habitats where A. anguilla grows and feeds have been greatly impacted by human activities, and the species is classified as “Critically Endangered” (Pike et al. 2020). In particular, the main reasons for its decline are thought to be related with the FW residency phase and the associated anthropogenic impacts on these systems, such as habitat modification and disruption, pollution, climate change, and overfishing (Drouineau et al. 2018; Pike et al. 2020). Various parasites and pathogens also represent a serious threat to European FW eels, including the introduced nematode Anguillicola crassus (Lefebvre et al. 2013; Drouineau et al. 2018). Therefore, to support effective management and conservation initiatives, it is critical to improve our understanding of A. anguilla trophic ecology. Using FA, SI, and elemental data from Norwegian eels, we examined the putative feeding habits of SW vs BW vs FW eels, and concomitant effects on eel condition. We also investigated the ecological advantages of habitat occupancy for the different life history strategies (i.e. FW/BW/SW residency vs habitat shifting) of A. anguilla. Specifically, as SW habitats typically provide fish with larger amounts of essential EPA and DHA, and energy-rich monounsaturated FA (MUFA; e.g. 20:1n-9 and 22:1n-11) than...
FW habitats (Parzanini et al. 2020), we hypothesized that a higher content of these FA would be positively correlated with eel size and condition for SW and BW eels compared to FW eels. In addition, we expected that fish and other higher trophic-level prey would represent the main food items of SW, i.e. that SW eels were feeding at higher trophic positions (compared to BW and FW eels).

**Materials and Methods**

**Sample collection**

Eels were collected in the summers of 2018 and 2019 from different sites in southern and western Norway (Figure 1). The sampling locations were selected to be representative of different salinity environments: SW, BW, and FW. Specifically, the SW sites were located in the euhaline coastal waters off the municipalities of Arendal and Grimstad (Skagerrak coast), and Bømlo (Bømlafjord). The BW stations were located nearby the mouth of the rivers Nivelda (Skagerrak coast) and Etneelva (Etnefjord), and within the inland and brackish water lake Landvikvannet (Skagerrak coast). This lake (1.85 km$^2$, maximum depth 25 m) is connected to the ocean by a 3 km long canal, which provides saltwater inputs following the tidal cycle. This creates a stratified system: the upper layer of the lake has low salinity (<15‰), while the salinity increases up to ~20-25‰, below 10 m depth (Eggers et al. 2014). Last, the FW site was located within the freshwater lake Litledalsvatnet, in the Etne municipality. Litledalsvatnet (0.95 km$^2$, maximum depth 63 m) has no tributaries, except for water going through a hydroelectric power plant and flowing into the lake at its eastern end. Litledalsvatnet is linked to the Etnefjord through the river Søreelva, which merges with the river Etneelva after ~6 km. A more detailed description of the sampling sites can be found as Supplementary Material.

Eels were caught using fyke nets (mesh size at the cod end was ~8 mm, knot-to-knot, and 11 mm along the diagonal) and eel pots (mesh size was ~10 mm, knot-to-knot, and 15 mm diagonal). The sex of all captured eels was determined by macroscopic observation of gonads.
to be female. Almost all eels were caught by authorized local fishermen. Eels in the Etne fjord were captured by some of the co-authors. Sampling and handling of eels in this study was approved by the Norwegian Animal Research Authority and all procedures followed local animal welfare regulations (FOTS id 15952).

A total of 233 individuals were anesthetized with clove oil and measured for total length (mm), wet mass (g) (Table S1), as well as eye diameter (mm) and fin length (mm). Eels were assigned a silvering stage following Durif et al. (2005) based on eye diameter, fin length, body length and wet mass. Specifically, stages I and II represent eels in their growth phase (classic “yellow” phase); while stage III indicates a pre-migrant phase, and stages IV and V are the two "silver" phases (Durif et al. 2005). Very few silver eels were caught (IV, n = 1; V, n = 2), and these were hence excluded from certain statistical tests, as specified below. Anesthetized eels were sacrificed and dissected. Skinless, white muscle tissue close to the dorsal fin (~1.0 x 0.5 cm) was collected for FA and SI analyses separately.

**Lipid extraction and FA analysis**

Muscle tissue samples were weighed and freeze-dried, after which the dry mass was recorded. Samples were then ground to fine powder. Lipids were extracted following a modified version of Folch et al. (1957) and quantified gravimetrically (total lipid content, TL; % dry weight, DW). FA were analysed as methyl esters (FAME) through gas chromatography using a Shimadzu GC-2010 Plus equipped with an AOC-20i auto-sampler and a flame ionization detector at Ryerson University, Toronto. Peaks were identified through comparison of retention times from various standards, including GLC-463 (Nu-Check Prep Inc.; FAME mix of 39 components), PUFA Mix No.1 (Supelco; marine FAME mix containing 22:1n-11, 11-docosenoic acid methyl ester), and 18:3n-4 (stearidonic acid; Sigma-Aldrich). Furthermore, FAME were quantified through calibration curves built on increasing concentrations of methylated 23:0 (methyl tricosanoate; Supelco), and GLC-68E (Nu-Check Prep Inc.; FAME mix of 20 components). In this study, FA
data are reported as percent (%) weights of FA sums and ratios. Specifically, the sums of saturated FA (SFA), MUFA, PUFA, n-3 and n-6 PUFA (hereafter, n-3 and n-6) were calculated along with the sum of EPA+DHA. The list of FA included in each sum is reported in Table S2. Moreover, the marine to freshwater (M/F; Parzanini et al. 2020), EPA/ARA, and n-3/n-6 ratios were calculated and used as indicators of feeding habitat (i.e. FW vs SW; Haliloğlu et al. 2004; Prigge et al. 2012). Specifically, the recently developed M/F ratio (Parzanini et al. 2020) provides indication of a predominantly marine- (M; higher values) vs freshwater-based diet (F; lower values), according to the presence of a few characteristic FA in fish muscle tissues (Table S2). We also used the 18:1n-9/18:1n-7 ratio as a biomarker for carnivory (Graeve et al. 1997).

**SI and elemental analysis**

Eel muscle tissues were removed and frozen on site before drying at 50°C for 24-48 h in a standard laboratory convection oven (Yamato DX 600, Yamato Scientific Company, Tokyo, Japan), and grinding to a homogenised powder using a mortar and pestle. The homogenate was used in SI (carbon, δ^{13}C, and nitrogen, δ^{15}N) and elemental analyses (carbon, %C, and nitrogen, %N) completed at the University of Waterloo Environmental Isotope Laboratory (UWEIL) using a dual-inlet and continuous-flow Delta Plus XL (Thermo-Finnigan, Germany) isotope-ratio mass spectrometer (IMRS) coupled to 4010 Elemental Analyser (CNSO 4010, Costech Analytical Technologies Inc., Valencia, USA), and with an analytical precision of ± 0.2‰ (δ^{13}C) and ± 0.3‰ (δ^{15}N). Analytical precision was determined by analysis of in-house laboratory standards cross-calibrated to the International Atomic Energy Agency standards CH6 for δ^{13}C and N1 and N2 for δ^{15}N run as controls throughout the analysis to ensure continued measurement accuracy. Elemental C and N, expressed as percent (%) composition of dry mass, were used to calculate elemental C to N ratios (C/N). Isotopic results were expressed using the standard delta notation (δ) as parts per thousand (‰) differences with respect to the...
international reference standards Vienna Pee Dee Belemnite and atmospheric nitrogen N\textsubscript{2} for
\(\delta^{13}\text{C}\) (Craig 1957) and \(\delta^{15}\text{N}\) (Mariotti 1983), respectively.

To avoid biases in the quantification of \(\delta^{13}\text{C}\) in lipid-rich tissues, as for eel muscles in this study, the mathematical correction by McConnaughey and McRoy (1979) was applied to calculate lipid-corrected \(\delta^{13}\text{C}’\) ratios. Detailed explanations of the rationale and procedure applied to calculate \(\delta^{13}\text{C}’\) are reported in Appendix 1. Similarly, to avoid biases in the interpretation of \(\delta^{15}\text{N}\) data due to the different baseline resources used to characterize the various habitats sampled in this study (e.g. Cabana and Rasmussen 1996), stable N isotope ratios were corrected (\(\delta^{15}\text{N}’\)) using the information provided by Østbye et al. (2018) (Appendix 2). Using the Østbye et al. (2018) data as proxies for our sites, the trophic position (TP) of eels was calculated using the equation provided by Cabana and Rasmussen (1996) (Appendix 2).

To assist in the interpretation of SI data across salinity zones, three metrics were calculated, following Layman et al. (2007). Specifically, the range of \(\delta^{13}\text{C}\) (CR) and \(\delta^{15}\text{N}\) (NR), which is the distance between the maximum and minimum stable C or N isotope ratios within each group of eels (i.e. FW, BW, and SW), were calculated together with the mean distance to centroid (CD). In this study, CD represents the average Euclidean distance of each FW, BW, or SW eel to the centroid (i.e. mean \(\delta^{13}\text{C}\) and \(\delta^{15}\text{N}\) for each eel group). Interpretation of these metrics was based upon relative differences across the three eel groups. While higher CR values indicate a more diversified diet (i.e. any given group of eels rely on more food sources), the higher the NR values of any given eel group the more trophic levels are encompassed, suggesting a higher degree of trophic variation within that group. Finally, the higher the CD values, the more dispersed the eels of any given group are, indicating a greater mean degree of trophic diversity for that group (Layman et al. 2007).
Statistical analysis

All of the continuous variables considered in this study (i.e. TL, SFA, MUFA, PUFA, n-3, n-6, EPA+DHA, EPA/ARA, n-3/n-6, M/F, 18:1n-9/18:1n-7, δ\textsuperscript{13}C, δ\textsuperscript{15}N, δ\textsuperscript{15}N', TP, C/N, body length, wet mass, and K\textsubscript{n}) were tested for normality and homogeneity of variance prior to statistical analysis. Where the assumptions of normality and homogeneity of variance were not met, non-parametric statistical tests were used.

Discriminant analysis was applied on standardized M/F, δ\textsuperscript{13}C', δ\textsuperscript{15}N, and C/N values to assess whether the biochemical composition of eels was a good predictor of the salinity of capture zones. In this study, we refer to biochemical composition as the composition of FA, SI, and/or elemental C and N characterizing eel muscle tissues. Raw δ\textsuperscript{15}N ratios were used instead of the corrected values to exploit the isotopic differences related to the characteristic baseline resources across SW, BW, and FW habitats. Permutational MANOVA (PERMANOVA) and principal coordinate analysis (PCoA) were used to test and visualize, respectively, the differences across eel groups in their biochemical composition. Specifically, a one-way PERMANOVA using salinity zone as the fixed factor, and TL and body length as covariates, was used to explore differences in the biochemical composition (i.e. normalized SFA, MUFA, PUFA, n-3/n-6, 18:1n-9/18:1n-7, δ\textsuperscript{13}C', δ\textsuperscript{15}N, C/N) among eel groups. Similarity percentage analysis (SIMPER) was performed on Euclidean distance to further analyze these differences, both within and between pairs of eel groups.

Kruskal-Wallis one-way analysis of variance on ranks (referred to as ANOVA on ranks, below) was used in conjunction with Dunn’s pairwise comparisons to determine significant differences in eel size (i.e. body length and wet mass), relative condition (K\textsubscript{n}, calculated using LeCren 1951), and biochemical composition (i.e. TL, SFA, MUFA, PUFA, EPA+DHA, n-3, n-6, EPA/ARA, n-3/n-6, M/F, 18:1n-9/18:1n-7, δ\textsuperscript{13}C', δ\textsuperscript{15}N, δ\textsuperscript{15}N', TP, and C/N) across salinity zones (i.e. SW, BW, and FW). To account for potential differences in body size, condition, and...
biochemical composition due to seasonal and ontogenetic variations, univariate statistics (the Student t-test, the non-parametric version represented by the Mann-Whitney rank sum test, and an ANOVA on ranks) were also performed between and among eel groups from different sampling years (i.e. 2018, 2019) and stages (i.e. I, II, III). Stages IV and V were excluded from this analysis, as they were represented by only 1 and 2 individuals per stage respectively.

Spearman rank correlations were used to assess the presence of significant relationships between eel size (i.e. body length and wet mass), condition ($K_\text{n}$) and biochemical composition (TL, SFA, MUFA, PUFA, EPA+DHA, n-3, n-6, M/F, EPA/ARA, n-3/n-6, 18:1n-9/18:1n-7, $\delta^{13}\text{C}'$, $\delta^{15}\text{N}'$, and C/N). The PERMANOVA, PCoA, and SIMPER were performed using PRIMER 7.0 with the PERMANOVA+ add on package, the discriminant analysis was run using Minitab 19, and univariate statistics (i.e. non-parametric ANOVA, post-hoc, and correlation tests) were carried out using SigmaPlot (version 12.5).

**Results**

Most eels (78%, $n = 181$ out of 233) were correctly classified by discriminant analysis into their salinity-habitat at point of capture using the variables, M/F, $\delta^{13}\text{C}'$, $\delta^{15}\text{N}'$, and C/N, thus making them good predictors of salinity-habitat. Misclassifications included $n = 35$ BW eels denoted as SW eels, and $n = 17$ SW eels labelled as BW eels. None of the FW eels were misclassified.

The FA, SI, and elemental composition of eel muscle varied significantly with salinity zone (PERMANOVA, Pseudo-$F = 54.7$, $p = 0.0001$), body length (Pseudo-$F = 71.0$, $p = 0.0001$), and TL (Pseudo-$F = 32.2$, $p = 0.0001$). Figure 2 shows these significant differences across SW, BW, and FW habitats; whereas Table 1 reports mean values ($\pm$ SD) of all the variables measured in SW, BW and FW eels. There were also significant interaction effects between body length and TL (Pseudo-$F = 7.7$, $p = 0.0001$), and between salinity zone and TL (Pseudo-$F = 2.3$, $p = 0.018$). The biochemical profiles of SW and BW eels were the most similar (SIMPER,
average square distance, $d^2 = 13.2$), whereas those of SW and FW were the most different ($d^2 = 28.4$). Specifically, the $\delta^{13}$C and $\delta^{15}$N ratios were the most important variables for discriminating between SW and FW eels and between BW and FW eels, but C/N, SFA, and MUFA drove the differences between BW and SW eels.

SW and BW eels had higher overall EPA+DHA contents (ANOVA on ranks, $H = 11.1, p = 0.004$), EPA/ARA ($H = 62.7, p \leq 0.001$), n-3/n-6 ($H = 53.8, p \leq 0.001$), M/F ($H = 77.0, p \leq 0.001$), 18:1n-9/18:1n-7 ($H = 14.3, p \leq 0.001$), and $\delta^{13}$C' ($H = 82.4, p \leq 0.001$) than their FW counterparts (Table 1). In addition, SW eels were characterized by the highest MUFA contents ($H = 40.3, p \leq 0.001$), $\delta^{15}$N' ratios ($H = 189.7, p \leq 0.001$), and TP ($H = 147.0, p \leq 0.001$), with mean TP values of $3.8 \pm 0.2$ (Figure 3). In contrast, FW eels were characterized by the highest PUFA contents ($H = 35.7, p \leq 0.001$), n-6 ($H = 68.1, p \leq 0.001$), TL ($H = 8.1, p = 0.017$). In addition, FW eels had a better condition value ($K_n = 1.1 \pm 0.1; H = 16.5, p \leq 0.001$) compared to BW ($K_n = 1.0 \pm 0.2$) and SW eels ($K_n = 1.0 \pm 0.2$), although the difference between FW and BW eels was not significant. While FW eels had the lowest TP ($2.9 \pm 0.3$), there was no significant difference with those of BW eels ($3.2 \pm 0.4$). However, BW eels had the most diverse diet ($CR = 7.8$) and highest trophic diversity ($NR = 5.9, CD = 1.8$) compared to FW ($CR = 4.2; NR = 4.7; CD = 1.5$) and SW eels ($CR = 6.5; NR = 4.6; CD = 1.4$).

Similar results were obtained when temporal and growth-stage variations were taken into account. Regardless of sampling year and/or stage, SW and BW eels had higher overall MUFA contents, as well as EPA/ARA, n-3/n-6, and M/F ratios in their muscle tissues than FW individuals, although differences were not always significant (Tables 2 & 3). In addition, SW eels had significantly higher stable C and N isotope ratios, and higher TP (Figure 3) than their BW and FW counterparts (Tables 2 & 3). In contrast, FW eels had significantly higher n-6 FA contents than BW or SW eels. No significant differences in TL content, body length, wet mass, and condition were observed across salinity zones, except for stage III-eels collected in 2018 where SW eels had higher TL contents and were larger in size ($p < 0.05$) than BW eels.
When eel muscle tissue data were analyzed to study differences across stages without considering the salinity factor, ANOVA on ranks detected several patterns based on the size, condition, and biochemical composition of the eels. Overall, later stage-eels had higher TL (ANOVA on ranks, \( H = 32.8, p \leq 0.001, 2018\)-eels; \( H = 9.8, p = 0.008, 2019\)-eels), MUFA (\( H = 34.5, p \leq 0.001, 2018\)-eels), and C/N contents (\( H = 25.2, p \leq 0.001, 2018\)-eels; \( H = 10.8, p = 0.005, 2019\)-eels). Figure 4 visualizes differences in the biochemical composition of eel muscle tissues across stages. This was also seen for their body length (\( p \leq 0.001\)), wet mass (\( p \leq 0.001\)), and condition (2018-eels, \( p = 0.006\); Table S4). In contrast, earlier-stage eels had higher PUFA contents (2018-eels, \( p \leq 0.001\)), n-3 FA (\( p \leq 0.001\)), and EPA+DHA (\( p \leq 0.001\)).

Size (i.e. body length and wet mass) and condition of eels were correlated with their biochemical composition (Spearman rank correlation; Table S5). Overall, body length and wet mass of the eels collected in both years were significantly correlated (\( p < 0.05\)) with their TL, MUFA, PUFA, and EPA+DHA contents, as well as C/N (Table S5). Similarly, in both years, eel condition was positively correlated with TL, C/N and EPA/ARA (\( p < 0.05\)).

**Discussion**

**Trophic ecology of European eels across salinity zones**

The combined use of FA, SI, and elemental biomarkers in this study provided insights into the trophic ecology and habitat use of a Norwegian population of European eels. The biochemical composition of eel muscle tissue varied significantly across salinity zones (i.e. SW, BW, and FW), as did total lipid content (TL) and body length, which is closely related to silvering stage (Durif et al. 2005). Moreover, our results suggest that the main biomarker differences among SW, BW, and FW eels are related to variations in their diet and feeding habits across habitats. Clear patterns were detected in the biochemical composition of eel muscle tissue throughout the various salinity zones, indicating that habitat has important effects.
As expected, the greatest biomarker differences were found between eels collected in SW vs FW habitats. Overall, SW eels were characterized by a greater content of FA that are indicators of a marine-based diet and feeding habitat (i.e. MUFA, EPA/ARA, M/F) and of carnivorous feeding behavior (18:1n-9/18:1n-7), and by higher SI ratios ($\delta^{13}$C', $\delta^{15}$N'). In contrast, FW eels exhibited the highest n-6 PUFA content, but had the lowest $\delta^{13}$C' and $\delta^{15}$N' ratios. Although still significantly different, the biochemical composition of BW eels was closer to SW eels than to FW eels. The discriminant analysis based on biochemical markers (i.e. M/F, $\delta^{13}$C', $\delta^{15}$N, and C/N) recognized 15% of BW eels as SW; and 7% of SW eels as BW eels. This could be due to: a) the availability of SW food sources within the BW sites selected for this study; b) the relatively small physical distance of the sampling stations between the two salinity zones; and/or, c) recent movements of some SW individuals into BW habitats, or vice versa.

Our results, based on isotopic and FA composition of eel muscle tissue collected in habitats that differ in salinity, are both similar and different from those of previous studies on European eel. For example, Harrod et al. (2005) examined the isotopic composition of European eels along a salinity gradient in Lough Ahalia, Galway (western Ireland) and found that the greatest differences in the stable C isotope composition were between SW (-16.3‰) and FW (-23.6‰) individuals, while BW eels exhibited ratios (-22.6‰) that were closer to the latter. However, they also observed that baseline-corrected $\delta^{15}$N ratios were equivalent in FW eels (5.8‰) and SW (5.8‰) eels, while those of BW individuals were significantly lower (4.8‰) suggesting BW eels were feeding at slightly lower trophic positions. In contrast, our analysis indicated that SW eels had higher $\delta^{15}$N' ratios, thus higher trophic position (TP), than eels in BW and FW. Regardless of this discrepancy, our results are consistent with the general consensus that organisms in FW ecosystems generally have lower $\delta^{15}$N relative to SW organisms (France 1994). This is attributed to the influence of low $\delta^{15}$N terrestrial inputs (typically < 7‰) to freshwater in contrast to the higher $\delta^{15}$N seen in oceanic POM (5 - 18‰) and marine plankton (Owens, 1988; Sharp 2007). Similarly, Vasconi et al. (2019) found that wild eels from marine
habitats had higher $\delta^{15}$N ratios than those collected from lagoons (raw data, 15.3 and 11.7‰, respectively), and similarly noted that lagoon eels had significantly higher content of n-6 PUFA than marine eels.

Along with the findings for TP based on $\delta^{15}$N, SI analysis indicated that SW eels occupied a higher TP than BW or FW eels, even when baseline- and size-related differences were taken into account. The higher levels of the FA carnivory index in SW compared to BW and FW individuals confirmed this result. Notably, the European eel is a bottom-feeder and a highly opportunistic predator (Bouchereau et al. 2009; Vasconi et al. 2019). As such, its diet can vary across seasons, life stages, sizes (Sinha and Jones 1967; Ezzat and El-Serrafy 1977; De Nie 1982), and head morphologies (Cucherousset et al. 2011; De Meyer et al. 2016). For instance, the diet of European eels from the upper zone of the Tagus Estuary shifted from amphipods and shore crabs to polychaetes, bivalves, and shrimps in more saline areas (Costa et al. 1992). Similarly, Ezzat and El-Serrafy (1977) and Sagen (1983) observed a size-related dietary shift of yellow European eels in Lake Manzalah (Egypt) and Austevoll (Norway), respectively. Whereas crustaceans, insect larvae, and molluscs were the main food items of smaller individuals, fish became a more important dietary item for larger eels (Ezzat and El-Serrafy 1977; Sagen 1983).

Although significant relationships between eel size and $\delta^{15}$N ratios were detected in this study, we did not find significant differences in size (i.e. body length and wet mass) among eels within each of the I, II, and III stages (except for stage III and III-eels collected in 2018) across salinity zones. Therefore, we cannot assess dietary shifts in relation to size variation across habitats of different salinity. Nonetheless, the overall higher levels of the carnivory index and TP detected in SW eels suggest that fish, and other higher trophic level-prey, are more important contributors to diet than for FW eels. Related to this, BW eels exhibited the largest isotopic ranges (i.e. CR and NR), and the highest mean distance to centroid CD. This suggests that BW eels in this study had access to a wider range of food resources compared to their FW and SW
counterparts, and were more omnivorous. Ultimately, the greater trophic diversity may be related to the general ability of eels to easily switch habitats (Dutil et al. 1988; Jessop et al. 2002).

**Condition of European eels across salinity zones**

As expected, body length and wet mass significantly increased with increasing stage, but a trend was also found across salinity zones. When eels were pooled together, independent of their stage, our analysis revealed that SW eels were larger compared to BW and FW eels. SW eels also had a higher MUFA content, which represents a main source of energy in fish (Tocher 2003). In contrast, FW eels were shorter, but had greater PUFA contents (mainly n-6 PUFA) than BW and SW eels, which is important as these represent high quality nutrients (Parrish 2009). Furthermore, FW eels had a slightly higher TL content, and were in better condition (Kn) than SW eels, contrary to our initial expectations.

As suggested by Gross et al. (1988), there might be an ecological (and therefore fitness) advantage to feeding and growing in FW rather than in BW and SW habitats that may apply for the FW eels analyzed in the current study. Despite the fact that SW and BW environments are more productive than FW ones at temperate latitudes (Gross et al. 1988), residing in FW habitats may allow eels to reduce the pressures of competition and predation (Tesch 1977; Moriarty 2003). Edeline et al. (2007) introduced the ‘conditional evolutionarily stable strategy’ model, according to which each eel can ‘choose’ any given strategy (e.g. FW phase, SW residency, and habitat shifting) that maximizes its fitness depending on its energetic status (e.g. body size) at any given time. For instance, yellow eels with larger body sizes may favor residency, whereas smaller eels may optimize their fitness by performing habitat shifting to avoid competition and reduce predation (Edeline et al. 2007). In this study, although FW eels had the smallest mean sizes, they were characterized by the highest content of TL and n-6 PUFA, both of which are critical for their maturation (see below). In contrast, SW eels had the
largest sizes and contents of MUFA, while their TL content was similar to that observed in FW eels, placing them in a favorable position with regard to the maturation process. Despite the fact that BW eels exhibited intermediate sizes, as well as TL, MUFA, and PUFA contents, their condition was comparable to that of their FW counterparts. This could potentially be explained by high trophic plasticity, allowing these eels to make habitat choices that maximize their fitness (Edeline 2007).

To achieve a more comprehensive understanding of the ecological advantages for European eels of living in a given salinity environment, future research should consider growth rate and age, eel parasite load, morphology (e.g. head, mouth gape), and/or competition and predation rates in the different habitats. All of these factors influence eel feeding behavior, growth, survival, and ultimately, fitness (Arai and Chino 2012). For example, mortality due to a high rate of parasitic infection has been cited as one of the main causes of the declines in FW eel populations (Lefebvre et al. 2013; Drouin et al. 2018). This might negate the better overall condition of FW eels relative to their SW counterparts. The analysis of the movement history of eels across habitats, as well as otolith chemistry and telemetry data, may also provide further clarification of these broader considerations.

The results obtained in this study indicated that SW, BW, and FW eels have different diets, which has implications for their FA profiles. SW and BW eels had higher MUFA and n-3 PUFA contents, as well as higher M/F, EPA+DHA, EPA/ARA, n-3/n-6, and 18:1-9/18:1-7 ratios, whereas FW were characterized by greater n-6 PUFA contents. Marine habitats are rich in long-chain n-3 PUFA (e.g. EPA and DHA), which are largely produced by phytoplankton and seaweeds (Dalsgaard et al. 2003; Parrish 2013). As most marine consumers cannot synthesize these FA de novo (at levels sufficient to meet their physiological needs), they have to acquire them through their diet (Parrish 2013). Thus, EPA and DHA are considered essential FA for optimal growth, reproduction, and survival in anguillid eels. (Bae et al. 2010; Baeza et al. 2014), with SW and BW individuals having a higher content of these two FA.
In contrast, the larger n-6 PUFA content in FW eels, which is generally observed in FW
fish species (Prigge et al. 2012; Parzanini et al. 2020), was most likely due to a large
contribution of the essential ARA in eel muscle tissue, as demonstrated by their significantly
lower EPA/ARA ratio. ARA is generally more abundant in FW vs SW fish (Parzanini et al. 2020).
Furthermore, ARA is a known key FA for both growth (Bae et al. 2010) and reproductive
success (Furuita et al. 2007; Støttrup et al. 2013; Kottmann et al. 2020) in anguillid eels. Not
only may FW eels acquire ARA through diet (e.g. FW molluscs are common eel prey items, De
Nie 1982; and are particularly rich in ARA, Wacker and von Elert 2004), but they also have the
capability to synthesize it from its n-6 precursor LNA (Kissil et al. 1987).

Variations across stages

Significant and noteworthy patterns in eel biochemical composition were also found in
association with increasing size and stage. Regardless of the salinity zone, later stage eels had
higher TL and MUFA contents, as well as higher C/N ratios, than earlier stage eels, whereas the
latter had a higher n-3 PUFA content (e.g. EPA+DHA). Lie et al. (1990) also reported an
increase in TL content with size in wild-caught eels. They also observed that larger individuals
had greater amounts of MUFA (50%), especially of 18:1n-9, but lower amounts of total PUFA
(~17%), compared to smaller eels (MUFA, ~44%; PUFA, ~25%). The size-related patterns for
TL and relative MUFA content observed here may be related to the accumulation of energy
stores (in the form of triacylglycerols) in eel muscle tissue prior to migration (Larsson et al. 1990;
Svedång and Wickström 1997). As mentioned above, these patterns were independent of the
salinity zone in which eels were caught, reinforcing the idea that factors other than diet should
be considered when characterizing examining eels’ ecology and habitat use. This result also
has implications for management and conservation. For instance, we suggest that the capture
of yellow eels with body lengths < 50 cm should be prohibited because those eels were
characterized by a content of < 20% DW, which is the critical threshold value for maturation and
migration success (Larsson et al. 1990; Svedäng and Wickström 1997). This would also
preclude the capture of male eels – since these are < 45 cm (Durif et al. 2005) – and are even
more at risk than females because of their lower proportion in the total population: male eels are
mainly localized to the southern part of the distribution area (Kettle et al. 2011).

Summary and conclusions

Habitat was a major driver of differences in the biochemical composition of muscle tissue in
Norwegian eels, and the main differences were observed between SW and FW individuals. SW
eels, collected from coastal waters, had a greater content of MUFA, n-3 PUFA (e.g. EPA+DHA),
carnivory index, and occupied the highest TP. In contrast, the FW eels sampled from a small
lake were characterized by the highest values of TL and n-6 PUFA, as well as condition. This
finding suggests major dietary differences between the two groups of eels, which are reflective
of the habitats in which each eel group feeds. Furthermore, whereas the biochemical
composition of BW eels was more similar to that of their SW counterparts, BW eels exhibited
the largest SI range, which suggests a high level of dietary plasticity. This large SI variation may
indicate the prevalence of habitat-shifting behaviors in BW eels, or could be due to the
availability of SW food sources within the BW sites selected for this study (i.e. river estuaries
and inland lake that receives inputs from the nearby sea). While it is still not possible to
conclude whether it would be more advantageous for eels to reside in FW or SW, or to adopt a
habitat-shifting strategy, the integration of different techniques to address this question, as in
this study, is key to achieving a greater understanding of this intricate, yet fascinating, “puzzle”.

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### Tables

**Table 1** Mean (± SD) total lipid content (TL) and composition, SI (δ^{13}C', δ^{15}N, base-line corrected δ^{15}N'), elemental (C/N) ratios, trophic position (TP), body size and condition (K_n) of European eels (*Anguilla anguilla*) collected across different salinity zones (sea = SW, brackish = BW, and freshwater = FW), with sample sizes. Lipid composition is represented by the sums of saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), omega-3 (n-3), omega-6 (n-6), eicosapentaenoic and docosahexaenoic (EPA+DHA) FA; and by the ratios of EPA and arachidonic acid (EPA/ARA), omega-3 and omega-6 FA (n-3/n-6), marine to freshwater FA (M/F), and 18:1n-9 and 18:1n-7 (18:1n-9/18:1n-7). The individual FA included in the sums of SFA, MUFA, PUFA, n-3, and n-6 can be found in Table S2. TL are reported in % DW; sums of FA as proportions (%), isotopic ratios in parts per thousand (‰); body length in millimeters (mm), and wet mass in grams (g). A letter code indicates significant differences (p < 0.05).

<table>
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<th>SW</th>
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<th>FW</th>
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<td>22.2 ± 12.3b</td>
<td>28.7 ± 15.2a</td>
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<td>PUFA</td>
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<td>n-3</td>
<td>20.0 ± 4.6</td>
<td>21.9 ± 5.8</td>
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<td>n-6</td>
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<td>6.1 ± 2.8a</td>
<td>11.3 ± 1.7b</td>
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<td>16.6 ± 5.7a</td>
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<td>M/F</td>
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<td>4.0 ± 1.7a</td>
<td>1.2 ± 0.3b</td>
</tr>
<tr>
<td>18:1n-9/18:1n-7</td>
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<td>-19.7 ± 1.8a</td>
<td>-28.3 ± 1.0b</td>
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<td>12.9 ± 0.8a</td>
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<td>δ^{15}N'</td>
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<td>TP</td>
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<td>C/N</td>
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<td>278.6 ± 199</td>
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<td>K_n</td>
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<td>1.0 ± 0.2b</td>
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Table 2 Mean (± SD) total lipid content (TL) and composition, SI (δ13C', δ15N, base-line corrected δ15N') and elemental (C/N) ratios, trophic position (TP), body size and condition (Kₙ) of European eels (A. anguilla) collected in summer 2018 across silvering stages (I, II, III, IV, and V) and salinity zones (sea = SW, brackish = BW, and freshwater = FW), with sample sizes. Lipid composition is represented by the sums of saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), omega-3 (n-3), omega-6 (n-6), eicosapentaenoic and docosahexaenoic (EPA+DHA) FA; and by the ratios of EPA and arachidonic acid (EPA/ARA), omega-3 and omega-6 FA (n-3/n-6), marine to freshwater FA (M/F), and 18:1n-9 and 18:1n-7 (18:1n-9/18:1n-7). The individual FA included in the sums of SFA, MUFA, PUFA, n-3, and n-6 can be found in Table S2. TL are reported in % DW; sums of FA as proportions (%), isotopic ratios in parts per thousand (‰); body length in millimeters (mm), and wet mass in grams (g). A letter code indicates significant differences (p < 0.05) across salinity zones, within each silverying stage.

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<th>V</th>
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<td>22.6 ± 4.0A</td>
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Table 3 Mean (± SD) total lipid content (TL) and composition, SI (δ¹³C, δ¹⁵N, δ¹⁵N') and elemental (C/N) ratios, trophic position (TP), body size and condition (Kₜ) of European eels (*A. anguilla*) collected in summer 2019 across silvering stages (I, II, III, IV, and V) and salinity zones (sea = SW, brackish = BW, and freshwater = FW), with sample sizes. Lipid composition is represented by the sums of saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), omega-3 (n-3), omega-6 (n-6), eicosapentaenoic and docosahexaenoic (EPA+DHA) FA; and by the ratios of EPA and arachidonic acid (EPA/ARA), omega-3 and omega-6 FA (n-3/n-6), marine to freshwater FA (M/F), and 18:1n-9 and 18:1n-7 (18:1n-9/18:1n-7). The individual FA included in the sums of SFA, MUFA, PUFA, n-3, and n-6 can be found in Table S2. TL are reported in % DW; sums of FA as proportions (%), isotopic ratios in parts per thousand (%); body length in millimeters (mm), and wet mass in grams (g). A letter code indicates significant differences (p < 0.05) across salinity zones, within each silvering stage.

<table>
<thead>
<tr>
<th>Variable</th>
<th>2019</th>
<th></th>
<th></th>
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<td>n = 26</td>
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<td>TL</td>
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<td>16.5 ± 5.2</td>
<td>17.6 ± 14.9</td>
<td>21.0 ± 11.7</td>
<td>25.0 ± 11.6</td>
<td>28.7 ± 13.3</td>
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<td>SFA</td>
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<td>MUFA</td>
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<td>36.3 ± 4.2</td>
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<td>41.3 ± 4.2A</td>
<td>37.9 ± 4.3B</td>
<td>36.4 ± 6.1</td>
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<td>PUFA</td>
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<td>32.9 ± 3.8</td>
<td>33.4 ± 3.7</td>
<td>28.3 ± 3.3A</td>
<td>28.4 ± 4.2A</td>
<td>31.8 ± 4.3B</td>
<td>32.4 ± 6.1</td>
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<tr>
<td>n-3</td>
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<td>29.0 ± 3.4b</td>
<td>21.3 ± 4.1a</td>
<td>23.3 ± 2.7a</td>
<td>24.0 ± 4.1A</td>
<td>20.5 ± 3.6B</td>
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<tr>
<td>n-6</td>
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<td>3.9 ± 0.7a</td>
<td>12.1 ± 1.5b</td>
<td>5.0 ± 1.4A</td>
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<td>11.2 ± 1.8B</td>
<td>5.7 ± 2.9</td>
</tr>
<tr>
<td>EPA+DHA</td>
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<td>23.9 ± 3.1b</td>
<td>14.4 ± 4.2a</td>
<td>18.2 ± 2.5A</td>
<td>18.9 ± 4.1A</td>
<td>12.8 ± 3.4B</td>
<td>22.2 ± 3.3</td>
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<td>EPA/ARA</td>
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<td>4.0 ± 0.9b</td>
<td>0.7 ± 0.2c</td>
<td>2.9 ± 1.3A</td>
<td>3.6 ± 1.0A</td>
<td>0.8 ± 0.2B</td>
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<td>n-3/n-6</td>
<td>5.8 ± 1.1a</td>
<td>7.5 ± 1.0b</td>
<td>1.8 ± 0.5c</td>
<td>5.0 ± 1.3A</td>
<td>6.0 ± 1.8A</td>
<td>1.9 ± 0.4B</td>
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<tr>
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<td>6.3 ± 0.6a</td>
<td>1.2 ± 0.4b</td>
<td>4.8 ± 1.3A</td>
<td>4.9 ± 1.4A</td>
<td>1.2 ± 0.3B</td>
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<tr>
<td>18:1n-9/18:1n-7</td>
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<td>7.3 ± 1.4a</td>
<td>4.7 ± 0.3b</td>
<td>5.5 ± 1.1A</td>
<td>7.4 ± 1.3B</td>
<td>4.7 ± 1.0C</td>
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<td>δ¹³C</td>
<td>-19.9 ± 0.5ab</td>
<td>-19.7 ± 1.5a</td>
<td>-27.2 ± 0.4b</td>
<td>-19.5 ± 0.8A</td>
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<td>11.6 ± 0.7b</td>
<td>8.3 ± 1.0c</td>
<td>12.6 ± 0.9A</td>
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<td>δ¹⁵N'</td>
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<td>3.8 ± 1.0b</td>
<td>6.3 ± 0.9A</td>
<td>3.8 ± 0.9B</td>
<td>2.8 ± 0.8C</td>
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<td>3.1 ± 0.3b</td>
<td>3.9 ± 0.3A</td>
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<td>C/N</td>
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<td>4.5 ± 0.9A</td>
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<td>Body length</td>
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<td>416.7 ± 26.2</td>
<td>407.5 ± 20.6</td>
<td>544.4 ± 58.7</td>
<td>524.2 ± 50.5</td>
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<tr>
<td>Wet mass</td>
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<td>134.8 ± 36.3</td>
<td>113.5 ± 27.8</td>
<td>273.7 ± 138.1</td>
<td>275.1 ± 98.5</td>
<td>249.7 ± 92.1</td>
<td>446.0 ± 93.3</td>
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<tr>
<td>$K_m$</td>
<td>1.1 ± 0.3</td>
<td>1.2 ± 0.1</td>
<td>1.1 ± 0.2</td>
<td>1.0 ± 0.2A</td>
<td>1.2 ± 0.2B</td>
<td>1.1 ± 0.1AB</td>
<td>1.1 ± 0.2</td>
</tr>
</tbody>
</table>
Figure 1 Sampling locations in southern and western Norway (a). Specifically, European eels (Anguilla anguilla) collected in summer 2018 were sampled along the Skagerrak coast (b); while individuals from the 2019 sampling season were collected within the county of Vestland (c). Refer to Table S1 for specific geographic coordinates of each sampling location. Map credit: C. Durif. Map created using the Manifold System software. Shapefiles for the base map were freely available and downloaded from the Norwegian Watershed and Energy Directorate (NVE) database (http://nedlasting.nve.no/gis/#).
Figure 2 Principal coordinates analysis (PCoA) plot showing differences in the fatty acid (saturated, SFA, monounsaturated, MUFA, and polyunsaturated FA, PUFA; ratio of omega-3 to omega-6 FA, n-3/n-6; carnivory index, 18:1n-9/18:1n-7), stable isotope ($\delta^{13}C$, $\delta^{15}N$), and elemental (C/N) composition of European eels (Anguilla anguilla) across sea- (SW), brackish (BW), and freshwater (FW) habitats. These differences were significant (PERMANOVA, Pseudo-F = 54.7, p = 0.0001). The plot was built on normalized data and on Euclidean distance-based matrices. The variance explained by principal coordinate 1 and 2 (PCO1 and PCO2) is reported in brackets. Linear vectors represent correlations of all the biochemical variables included in the analysis with the ordination axes. The closer to the perimeter of the circle these linear vectors are, the higher the correlation.
Figure 3 Trophic positions (TP) of European eels (*Anguilla anguilla*) across different salinity zones (sea = SW, brackish = BW, and freshwater = FW). TP were calculated using the formula by Cabana and Rasmussen (1996), while isotopic information of potential food sources was provided by Østbye et al. (2018).
Figure 4 Differences in the fatty acid (saturated, SFA, monounsaturated, MUFA, and polyunsaturated FA, PUFA; ratio of omega-3 to omega-6 FA, n-3/n-6; carnivory index, 18:1n-9/18:1n-7), stable isotope (δ^{13}C', δ^{15}N), and elemental (C/N) composition of European eels (Anguilla anguilla) across different silvering stages plotted through principal coordinates analysis (PCoA). The plot was built on normalized data and on Euclidean distance-based matrices. The variance explained by principal coordinate 1 and 2 (PCO1 and PCO2) is reported in brackets. Linear vectors represent correlations of all the biochemical variables included in the analysis with the ordination axes. The closer to the perimeter of the circle these linear vectors are, the higher the correlation.
Appendices

Appendix 1. Rationale behind the mathematical correction of stable C isotope ratios (δ¹³C)

Due to the preferential discrimination for the lighter C isotope (i.e. ¹²C) during lipid synthesis (DeNiro and Epstein 1977), lipid-rich tissues (e.g. fish white muscle) may display lower δ¹³C ratios, leading to biases in their quantification (McConnaughey and McRoy 1979; Post et al. 2007). For this reason, mathematical corrections based on total lipid (TL) content, or bulk C/N ratio, as a proxy for TL can be applied (McConnaughey and McRoy 1979; Post et al. 2007). In general, C/N ratios >3.5 indicate high TL contents (i.e. >5% DW), thus a mathematical correction is recommended (e.g. Post et al. 2007). European eels (Anguilla anguilla) are considered fatty fish, and we found that mean TL content of eel muscle tissue were 25.0 ± 13.9% DW (n = 233) based on gravimetric analysis, while their mean C/N ratios were 5.0 ± 1.3 (n = 233). A Spearman rank correlation test was therefore performed to assess whether there was a significant relationship between TL and δ¹³C, and between TL content and C/N, as a prerequisite for mathematical correction (Post et al. 2007). Our results indicated a significant relationship between TL and δ¹³C (rₛ = -0.4, p < 0.001), as well as between TL and C/N (rₛ = 0.9, p < 0.001). Therefore, the following equation from McConnaughey and McRoy (1979) was applied:

\[ \delta^{13}C' = \delta^{13}C + D \times \left[ -0.207 + 3.90 / (1 + 287 / TL) \right] \]

where δ¹³C' and δ¹³C are the lipid-corrected and the raw ratios of stable C isotope; D is the isotopic difference between proteins and lipids, which was calculated as 6‰ by McConnaughey and McRoy (1979); TL is the total lipid content of a given sample. Note that, in this study, TL content was gravimetrically established, and was not derived indirectly from C/N values as in McConnaughey and McRoy (1979). The difference between lipid-corrected and raw data (Δδ¹³C) was regressed against TL, and the model explained 99.8% of the variability (\( \Delta \delta^{13}C = -1.0012 + 0.067648 \times TL \)). Overall, mathematical correction significantly increased δ¹³C data by 0.6‰ (Mann-Whitney rank sum test, U = 23019, p = 0.001). Nonetheless, in a few cases (n = 78 out of 233, i.e. 33%) the values of the ratio decreased; in those instances, samples were characterized by C/N values <4.
Appendix 2. Rationale behind the mathematical correction of stable N isotope ratios ($\delta^{15}N$)

To avoid biases in the interpretation of $\delta^{15}N$ data due to the different baseline resources used to characterize the various habitats sampled in this study (e.g. Cabana and Rasmussen 1996), stable N isotope ratios were corrected ($\delta^{15}N'$) using the information provided by Østbye et al. (2018). These authors analyzed the isotopic composition of several organisms collected in environments similar to those in our study, specifically, along a salinity gradient in the Oslofjord Fjord (south-eastern Norway). These organisms are known as potential prey items of European eel (Anguilla anguilla), including Chironomidae larvae, polychaetes, shrimp, the blue mussel (Mytilus edulis) and the three-spined stickleback (Gasterosteus aculeatus). Using the Østbye et al. (2018) data as proxies for our sites the trophic position (TP) of eels was calculated following Cabana and Rasmussen (1996), as:

$$TP = \left[ \frac{\delta^{15}N - \delta^{15}N_{\text{base}}}{\Delta^{15}N} \right] + \text{TP}_{\text{base}}$$

where $\delta^{15}N$ and $\delta^{15}N_{\text{base}}$ are the stable N isotope ratios of eels (uncorrected) and prey, respectively, $\Delta^{15}N$ is the fractionation factor, and $\text{TP}_{\text{base}}$ is the trophic position of the prey item. In this study, we used a fractionation factor of 3.4‰, as applicable to a large variety of organisms (Minagawa and Wada 1984; Cabana and Rasmussen 1996). FW, BW, and SW $\delta^{15}N_{\text{base}}$ ratios (all with $\text{TP}_{\text{base}} = 2$) were obtained from Østbye et al. (2018) and consisted of “Chironomidae upper” (4.5‰), “Gammarus lower” (7.9‰), and “blue mussel” (6.6‰) respectively. In Østbye et al. (2018), “upper” and “lower” refer to the specific sections of Lake Engervann, where prey-item samples were collected.

References


