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Feeding habitat and silvering stage affect lipid content and fatty acid composition of European eel *Anguilla anguilla* tissues

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Abstract

Lipids, particularly fatty acids (FAs), are major sources of energy and nutrients in aquatic ecosystems and play key roles during vertebrate development. The European eel *Anguilla anguilla* goes through major biochemical and physiological changes throughout its lifecycle as it inhabits sea- (SW), and/or brackish- (BW) and/or freshwater (FW) habitats. With the ultimate goal being to understand the reasons for eels adopting a certain life history strategy (FW or SW residency *vs.* 'habitat shifting'), we explored differences in lipid content and FA composition of muscle, liver and eyes from eels collected across Norwegian SW, BW and FW habitats, and at different lifecycle stages (yellow to silver). FW and SW eels had a higher lipid content overall compared to BW eels, reflecting differences in food availability and life history strategies. SW eels had higher proportions of certain monounsaturated FAs (MUFAs; 18:1n-9, 20:1n-9), and of the essential polyunsaturated FAs 20:5n-3 (eicosapentaenoic acid, EPA) and 22:6n-3 (docosahexaenoic acid) than FW eels, reflecting a marine-based diet. In contrast, the muscle of FW eels had higher proportions of 18:3n-3, 18:2n-6 and 20:4n-6 (arachidonic acid), as is typical of FW organisms. MUFA proportions increased in later stage eels, consistent with the hypothesis that the eels accumulate energy stores prior to migration. In addition, the decrease of EPA with advancing stage may be associated with the critical role that this FA plays in eel sexual development. Lipid and FA information provided further understanding of the habitat use and overall ecology of this critically endangered species.

KEYWORDS
ARA, DHA, EPA, facultatively catadromous, habitat shifting, salinity

1 | INTRODUCTION

During their continental life, juvenile European eels *Anguilla anguilla* L. 1758 go through a period of growth prior to maturation and migration back to the Sargasso Sea (Durif *et al.*, 2005; Moriarty, 2003; Tesch, 1977). Yellow eels (*i.e.*, immature eels) spend up to 30 years or more in continental waters (Durif *et al.*, 2009a, 2020; Poole & Reynolds, 1996), including in sea (SW), brackish (BW) and fresh water (FW) to feed and grow (Moriarty, 2003). While moving across SW, BW and FW habitats (Daverat *et al.*, 2006), yellow eels undergo considerable physiological changes that allow them to cope with variations in salinity (Kalujnaia *et al.*, 2007; Skadhauge, 1969; Thomson & Sargent, 1977) and other environmental conditions (*e.g.*, temperature, White & Knights, 1997). Typically, glass eels or elvers enter FW and swim upstream into rivers and lakes (Tesch, 1977; Tzeng *et al.*, 2000). Once they accumulate sufficient energy stores as yellow eels

(*i.e.*, >20% muscle fat content; Boëtius & Boëtius, 1980; Larsson *et al.*, 1990; Svedäng & Wickström, 1997), they go through a pre-pubertal event termed 'silvering' and migrate back to the Sargasso Sea (Aroua *et al.*, 2005; Dufour *et al.*, 2003; Durif *et al.*, 2005). As a facultatively catadromous species (Daverat *et al.*, 2006), eels may remain in SW or FW habitats to grow, or move back and forth between SW and FW, that is, they may employ a 'habitat-shifting' strategy (Daverat *et al.*, 2006).

The traditional classification of yellow and silver eels (*i.e.*, maturing adults) is mainly based on external morphological traits, including body colouration and/or the size of the eyes and pectoral fins (Durif *et al.*, 2005; Pankhurst, 1982). Through multivariate statistical techniques, Durif *et al.* (2005, 2009b) combined external and internal morphological traits (*e.g.*, gut and gonad mass) with physiological indicators (*e.g.*, gonado-somatic and hepato-somatic indices, and gonatropin levels) to provide a more comprehensive evaluation of the features characterizing the different life stages in *A. anguilla*. They were able to discriminate five different silvering stages for females, such as stages I and II, which correspond to a growth phase; stage III, or a pre-migrant, preparatory phase; and stages IV and V, which both represent a migrating phase (Durif *et al.*, 2009b).

Migrating eels (*i.e.*, stages IV and V) typically have larger body and eye sizes, and higher energy reserves (mainly in the form of lipids) compared to earlier stages (Beullens *et al.*, 1997; Boëtius & Boëtius, 1980; Pankhurst, 1982). When eels stop feeding, their guts begin to degenerate (Durif *et al.*, 2005; Tesch, 1977) and the energy accumulated during the growth phase is partly directed towards gonad and gamete development and sexual maturation, although this process is only completed during migration (Palstra & van den Thillart, 2010; Pierron *et al.*, 2007). Energy reserves support the final migration to the Sargasso Sea (Palstra & van den Thillart, 2010) and subsequent spawning activity (Svedäng & Wickström, 1997). Palstra and van den Thillart (2010) calculated that silver *A. anguilla* invest ~70% of their stored energy in the spawning migration and oocyte maturation. When environmental conditions (*e.g.*, temperature, luminosity, water turbidity; Vøllestad *et al.*, 1986; Durif *et al.*, 2003) are not favourable, eels may abandon the migratory phase and revert to the growth phase (Durif *et al.*, 2005). Thus, it is important to gain a more complete understanding of energy storage and nutrient acquisition, from the perspective of lipids, in eels.

Lipids are the densest form of energy in aquatic ecosystems, and certain lipids, including several long-chain polyunsaturated fatty acids (LC-PUFAs), provide consumers with essential nutrients (Arts *et al.*, 2001; Parrish, 2009, 2013). Among these, 20:5n-3 (eicosapentaenoic acid, EPA), 22:6n-3 (docosahexaenoic, DHA), and 20:4n-6 (arachidonic, ARA) are the most important nutrients in aquatic ecosystems (Parrish, 2013). The term 'essential' assigned to these FAs (*i.e.*, EFAs) originates from the fact that these nutrients support multiple key physiological processes, and cannot be synthesized at all, or at rates sufficient to meet demand. Hence, these compounds must be acquired through diet by aquatic organisms for optimal growth, reproduction and survival (Arts *et al.*, 2001; Parrish, 2009). Dietary ARA, for instance, promotes normal growth in juveniles of Japanese eel *A. japonica* Temminck & Schlegel 1846 (Bae *et al.*, 2010), while EPA in the liver of *A. anguilla* plays a key role in promoting gamete production and quality (Baeza *et al.*, 2014; Butts *et al.*, 2015). Similarly, Butts *et al.* (2015) suggested that the production of milt in farmed *A. anguilla* is mostly dependent on dietary DHA, with higher volumes of extractable milt associated with higher levels of DHA. As DHA is a key component of the eye retina (Mourente, 2003), dietary deficiencies in this EFA reduced visual acuity in juveniles of Atlantic herring *Clupea harengus* L. 1758, especially under low-light conditions (Bell *et al.*, 1995).

Specific FAs are also regarded as dietary biomarkers (Arts *et al.*, 2001; Dalsgaard *et al.*, 2003; Napolitano, 1999), and the FA composition of consumers typically reflects that of their diet (Dalsgaard *et al.*, 2003; Napolitano, 1999) and feeding habitat (Parzanini *et al.*, 2020). Eel diets in habitats characterized by different salinities may be mirrored accordingly in their FA profiles (Prigge *et al.*, 2012). However, lipid content and the FA profiles of different tissues and organs may also vary according to their specific physiological roles.

Muscle and liver are the main fat (primarily in the form of triacylglycerols, TAG) storage tissues in Anguillidae (Boëtius & Boëtius, 1985; Pierron *et al.*, 2007). In wild *A. anguilla*, the TAG constitute 90% and 40% of total lipid composition of muscle and liver tissue, respectively (Burgos *et al.*, 1989). In addition, the liver is the main site of lipogenesis (Tocher, 2003) and energy turnover (Hansen & Abraham, 1983) in fish. Lipids ingested through the diet can either be rapidly oxidized to produce metabolic energy (*i.e.*, adenosine triphosphate, ATP), or converted into structural (*e.g.*, phospholipids, PL) or storage (*e.g.*, TAG) lipids in the liver (Tocher, 2003). During vitellogenesis, the liver relocates the lipid reserves mobilized from the muscle into eggs *via* egg-specific lipoproteins (*i.e.*, vitellogenin; Sargent *et al.*, 1989; Tocher, 2003; Ozaki *et al.*, 2008).

Eyes have been used as indicators of sexual maturity in *A. anguilla* (Beullens *et al.*, 1997; Pankhurst, 1982). Pankhurst (1982) described correlations between body length, gonad development and eye diameter in female *A. anguilla*, and developed an 'eye index' to discriminate between immature (*i.e.*, yellow, eye index ≤6.5) and maturing (*i.e.*, silver, eye index >6.5) female individuals. Pankhurst (1982) also noted that the diameter of the outer segments of the rods and the total amount of photoreceptors increased with maturity (*i.e.*, silvering). Both structural and visual changes occur in *A. anguilla* during silvering (Wald, 1960), and both are completed prior to migration. Such changes are thought to prepare eels for their SW migration and subsequent spawning (Pankhurst, 1982). In this context, increasing the amount of certain FAs, including DHA, in eel eyes may be critical.

As lipids are a major form of energy and essential nutrients in aquatic ecosystems (Parrish, 2009, 2013), variations in the quantity and quality of these critical compounds across habitats characterized by different salinities may have implications for eel growth, maturation and migration to the Sargasso Sea. Because *A. anguilla* is 'Critically Endangered' according to the IUCN Red List (Pike *et al.*, 2020), improving our knowledge of eel growth and eel dietary and maturation requirements is important. Understanding which habitat (SW *vs.*

BW *vs.* FW) and combination thereof provides the most abundant and/or best-quality nutrients may help us understand the relative benefits for eels of spending time in each. This information contributes to the development of conservation and management strategies for the species.

In this study, we explored differences in the lipid content of muscle, liver and eyes of *A. anguilla* collected in Norway by quantifying variation in the composition of individual FAs that are energetically, structurally and functionally important. We examined variations in total lipid content and FA composition of three key eel tissues/organs across different aquatic habitats (*i.e.*, SW, BW and FW) and silvering stages (*i.e.*, I to V). We hypothesized that (a) regardless of the stage, the muscle FA profiles of *A. anguilla* would reflect that of their feeding habitat(s), as previously observed for fish in general by Parzanini *et al.*, (2020), and (b) eye FA profiles would be predictive of the maturation level irrespective of habitat (SW, BW and/or FW). We further hypothesized that (c) energy reserves, in terms of total lipid content and monounsaturated FAs (MUFAs), would increase with advancing stage, especially in eel muscle given its role as energy storage site, and (d) the content of DHA would increase in eel eyes as they matured.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Eels ($n = 233$) representing different salinity zones and silvering stages (Figure 1) were collected from various coastal locations in southern and western Norway during August 2018, and July and September 2019. Eels were caught using fyke nets (cod end mesh size ~8 mm knot-to-knot and 11 mm along the diagonal) and traps (mesh size ~10 mm knot-to-knot and 15 mm diagonal) deployed by authorized local fishermen. The study was approved by the Norwegian Animal Research Authority and performed in accordance with animal welfare regulations (FOTS id 15,952). All of the eels caught were females, based on macroscopic observation of the gonads during sampling. Details of sample locations and collection, as well as salinity at the point of capture, are presented in Supporting Information Table S1. Information on sample sizes (*n*) and eel measurements (*e.g.*, total body length in mm and wet mass in g) across differing salinity zones and silvering stages is presented in Supporting Information Table S2. Measurements of eye diameter, fin and body length, and wet mass were used to assign a silvering stage to eels, following Durif *et al.* (2005, 2009b).

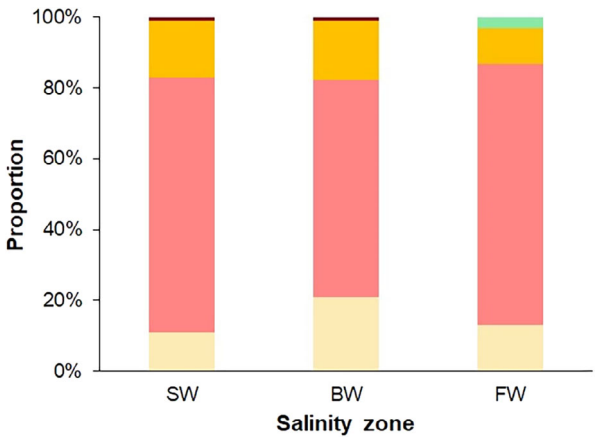

FIGURE 1 Proportion (%) of European eel *Anguilla anguilla* representing each silvering stage (*i.e.*, I, II, III, IV, V) by salinity zone (*i.e.*, BW, brackish water; FW, freshwater; SW, seawater). (□) I; (□) II; (□) III; (□) IV; (■) V

Biopsies from three different tissues/organs were taken from each individual eel for lipid analysis: skinless white dorsal muscle tissue drawn from either side of the dorsal fin (~1.0 × 0.5 × 0.5 cm^3 pieces of tissue), liver (~1.0 × 1.0 × 0.5 cm^3 pieces of tissue) and the two eyes. These samples were stored in separate cryovials and frozen at −20°C for a maximum of 5 days prior to transfer to the Institute of Marine Research's facility in Austevoll, where they were then kept at −80°C for ~1 week. The samples were then shipped, on dry ice, to Ryerson University in Toronto, Canada, where they were stored at −80°C until they were analysed.

2.2 | Lipid extraction and fatty acid methylation

Lipids were extracted using a modified version of the Folch *et al.* (1957) method in a chloroform:methanol (2:1) solution, and the FAs analysed as methyl esters (FAME). Prior to lipid extraction, samples were freeze-dried and their dry weights recorded. Aliquots of ~5–30 mg of dry mass were weighed and transferred to lipid-clean test tubes. A 4 ml aliquot of chloroform:methanol solution was added to each sample, along with 0.8 ml of KCl (0.88%) to remove impurities. Samples were vortexed and then centrifuged for 5 min at 2000 rpm and 4°C to allow the separation of two layers. The bottom layer, consisting of the chloroform:methanol lipid extract, was pipetted out into new clean tubes, while 2 ml of the chloroform:methanol:water (86:14:1) solution was added to the tubes containing the KCl solution. These tubes were vortexed again and the extraction repeated three times. The final extracts were evaporated down using a gentle stream of N_2. After evaporation, 2 ml of hexanes was added to the tubes and the tubes were vortexed. A volume of 0.1 ml of the lipid extracts was pipetted into pre-weighed seamless tin cups, and the solvents were allowed to evaporate overnight. The tin cups were then weighed again and the weight difference was used for the evaluation of total lipid content computed as a percentage of the dry weight of tissue extracted (% DW).

FAs were methylated by adding 2 ml of H_2SO_4 in methanol (1% v/v) to each sample. The tubes were then vortexed, sealed under N_2 and placed on a heat block at 90°C for 90 min. Once cooled, 1.5 ml of Milli-Q water and 4 ml of hexanes were added, and the samples were centrifuged at 2000 rpm and 4°C for 3 min, leading to the formation of two separate layers. The top organic layer was transferred into new lipid-clean tubes, and the last few steps were repeated three times to remove all the water from the samples. The final FAME extracts were evaporated down using N_2. Once

evaporated, a known volume (200–750 μl) of hexanes was added to the tubes, which were centrifuged, and the solution was transferred into labelled chromatography vials.

The FAME extracts were analysed by gas chromatography (GC; split injection ratio 50:1, two lines), with a Shimadzu GC-2010 Plus equipped with an AOC-20i auto-sampler and a flame ionization detector (FID). Two SP-2560 columns (100 m × 0.25 mm × 0.2 μm; Supelco Inc., Bellefonte, PA) were used. Peaks were identified by comparing retention times from standards, including a 39-component FAME mix (Nu-Check Prep Inc., Elysian, MI, catalogue# GLC-463), a marine PUFA mix inclusive of 22:1n-11 (11-docosenoic acid methyl ester; Supelco Inc., Bellefonte, PA, product name PUFA Mix No.1) and 18:4n-3 (stearidonic acid; Sigma-Aldrich, Oakville, ON Canada, catalogue# SMB00291). Furthermore, FAs were quantified by comparing the retention times of a 20-component FAME mix (Nu-Check Prep Inc., catalogue# GLC-68E). In this study, individual FA data were reported as percentage weights (%) of total identified FAs, *i.e.*, as relative proportions of individual FAs. Furthermore, here FAs were expressed following the A:Bn-X notation, where A represents the number of carbon atoms, B is the number of double bonds, and X is the position of the first double bond from the methyl end.

2.3 | Statistical analysis

Only individual FAs present with mean proportions ≥0.5% across the different tissue/organs (*i.e.*, muscle, liver and eye) were analysed statistically (*i.e.*, $n = 17$ out 46 FAs; Table 1) as these were assumed to contribute most to the variability. Further, FA data were arcsine square root transformed to meet the assumptions of normality and homogeneity of variance. In the case of total lipid content, raw (untransformed) data were used for analysis.

One-way permutational MANOVA (PERMANOVA) and principal coordinates analysis (PCoA) were run to assess and visualize,

TABLE 1 Mean (± S.D.) total lipid content (% DW) and fatty acid FA composition (%), and sample sizes for eye, liver and white muscle of European eel *Anguilla anguilla*

FA		Muscle	Liver	Eye
Formula	**Name**	***n* = 233**	***n* = 233**	***n* = 227**
Total lipids		25.0 ± 13.9	23.2 ± 6.9	9.6 ± 4.6
Saturated				
14:0	Myristic acid	4.2 ± 0.9	1.7 ± 0.5	1.7 ± 0.6
16:0	Palmitic acid	20.1 ± 1.3	20.0 ± 2.5	18.8 ± 1.7
18:0	Stearic acid	4.5 ± 1.0	8.5 ± 1.7	10.1 ± 1.8
Monounsaturated				
16:1n-7	Palmitoleic acid	7.2 ± 1.8	3.9 ± 1.3	3.1 ± 1.2
17:1n-7	Heptadecenoic acid	0.6 ± 0.3	0.4 ± 0.2	1.0 ± 0.4
18:1n-9	Oleic acid	25.8 ± 5.0	19.7 ± 8.4	12.7 ± 3.8
18:1n-7	Vaccenic acid	4.8 ± 1.2	4.3 ± 1.4	3.0 ± 0.8
20:1n-9	Gondoic acid	1.4 ± 0.9	1.2 ± 0.5	0.7 ± 0.4
24:1n-9	Nervonic acid	0.2 ± 0.1	0.6 ± 0.2	0.7 ± 0.3
Polyunsaturated				
Omega-6				
18:2n-6	Linoleic acid	1.7 ± 1.2	1.1 ± 0.6	0.7 ± 0.6
20:2n-6	Eicosadienoic acid	0.6 ± 0.3	0.7 ± 0.4	0.4 ± 0.3
20:4n-6	Arachidonic acid (ARA)	2.5 ± 1.6	6.2 ± 2.7	4.8 ± 1.5
22:4n-6	Adrenic acid	1.0 ± 0.8	2.0 ± 1.5	1.4 ± 0.8
Omega-3				
18:3n-3	α-Linolenic acid	1.4 ± 0.9	0.7 ± 0.4	0.6 ± 0.5
20:5n-3	Eicosapentaenoic acid (EPA)	4.6 ± 1.0	5.2 ± 1.5	3.2 ± 1.0
22:5n-3	Clupanodonic acid	3.1 ± 0.9	3.4 ± 1.3	2.3 ± 0.7
22:6n-3	Docosahexaenoic acid (DHA)	11.0 ± 4.7	15.8 ± 5.1	31.9 ± 7.2
Minor FA fraction[a]		5.3 ± 1.5	4.5 ± 1.3	3.1 ± 1.1

Note. Only FAs with mean proportions ≥0.5% across tissues (*i.e.*, $n = 17$ out of 46 detected) were considered in this analysis. Mean total fractions of the FAs excluded are also reported.

[a]Included in this fraction were the following FAs: 14:1n-5, *i*15:0, 15:0, 15:1n-5, 16:1n-9, 16:1n-7 *t*, 17:0, 18:1n-9 *t*, 18:1n-12, 18:1n-7 *t*, 19:0, 19:1n-12, 20:0, 18:3n-6, 20:1n-15, 20:1, 18:2n-6 *t*, 20:1n-11, 18:4n-3, 22:3n-3, 22:0, 20:3n-6, 22:1n-11, 22:1n-9, 20:3n-3, 20:4n-3, 22:2n-6, 24:0, 22:5n-6. The letter *i* indicates *iso*-branched FAs, whereas *t* refers to the *trans* -configuration of the molecule.

respectively, differences among tissues/organs. In addition, similarity percentage analysis (SIMPER) was performed to assess similarities within tissues/organs. Distance-based linear model (DistLM) analysis was run on resemblance matrices built from transformed individual FA data and based on Euclidean distances to assess which environmental (*i.e.*, salinity zone, sampling year) and/or biological (*i.e.*, stage, tissue/organ type, total length and total lipid content) variable(s) contributed most to the variability in FA composition. Individual eel weight was excluded from the analysis as this measure showed a high positive correlation with length and was hence redundant. Based on DistLM results, further analyses were carried out targeting each tissue/organ separately. Specifically, two-way crossed PERMANOVA tests were run to explore the effects of salinity zone, stage and their interaction on the FA composition of each tissue/organ, along with SIMPER to study dissimilarities of specific tissue/organ FA profiles across salinities. PERMANOVA tests were conducted on Euclidean distance-based matrices with the 'unrestricted permutation of raw data' method (number of permutations 9999; type III sums of square), whereas SIMPER was done on Bray–Curtis similarities.

Based on the results obtained from multivariate analysis, one-way analysis of variance (ANOVA) and/or a Kruskal-Wallis ANOVA on rank data (below, ANOVA on ranks; nonparametric test) were run to further investigate differences in total lipid content and FA composition across and within tissues/organs. Significant results were investigated further using Holm-Sidak and Dunn's (nonparametric test) pairwise comparisons. Given the unbalanced data set, and as a further assessment of lipid content and composition differences across salinity zones, ANOVA tests were repeated considering stage II eels only. This stage is typically the most common as it represents yellow eels with an average length of 50 cm. Only eels from this stage were equally represented in this dataset and had a sufficient number of representatives from each salinity zone on which to base an analysis ($n_{SW} = 22$, $n_{BW} = 74$, $n_{FW} = 60$). As a supporting analysis of differences across stages, additional ANOVA tests were run using BW eels only, as they had the highest sample sizes representing stages I to III ($n_{I} = 5$, $n_{II} = 28$, $n_{III} = 14$). Stages IV and V were excluded from the statistical analyses due to low sample size ($n_{IV} = 1$ and $n_{V} = 2$). Lastly, Spearman rank order correlation analysis was performed to determine the significance of any correlation between eel size (*i.e.*, total length and mass) and the lipid content of each tissue/organ. Multivariate (*i.e.*, DistLM, PERMANOVA, SIMPER and PCoA) and univariate statistics (*i.e.*, ANOVA on ranks, post-hoc and Spearman correlation tests) were performed using PRIMER 7.0 and the PERMANOVA+ add on package, and SigmaPlot (ver. 12.5), respectively.

3 | RESULTS

3.1 | Overall lipid content and FA composition of *A. anguilla* tissues and organs

The total lipid content of eyes was significantly lower than that of muscle and liver (ANOVA on ranks, $H = 320.3$, $P \leq 0.001$) (Table 1).

The FAs present with the greatest mean proportions (± S.D.) across the three tissues were 16:0 (19.6 ± 2.0%), DHA (19.5 ± 10.6%) and 18:1n-9 (19.4 ± 8.1%). The remaining FAs represented <8% of the total. While the FA composition was significantly different among the three tissues/organs (PERMANOVA, pseudo-$F = 378.1$, $P = 0.0001$; Figure 2), the average similarity within each tissue/organ was ≥90% (SIMPER). Muscle was characterized by 18:1n-9 (25.8 ± 5.0%), followed by 16:0 (20.1 ± 1.3%), DHA (11.0 ± 4.7%), 16:1n-7 (7.2 ± 1.8%) and EPA (4.6 ± 1.0%) (Table 1). The main contributors (> 50%) of the similarity within eye samples were DHA (31.9 ± 7.2%), followed by 16:0 (18.8 ± 1.7%), 18:1n-9 (12.7 ± 3.8%) and 18:0 (10.1 ± 1.8%; Table 1), while 16:0 (20.0 ± 2.5%), 18:1n-9 (19.7 ± 8.4%), DHA (15.8 ± 5.1%), 18:0 (8.5 ± 1.7%) and ARA (6.2 ± 2.7%) were the most important FAs in liver.

All the predictors considered here contributed significantly to explaining variability in eel FA composition, with 'tissue/organ type' being the greatest contributor (52.3%), followed by total lipid content (7.6%), salinity zone (3.9%), total length (3.5%), year (2.7%) and stage (2.5%) (DistLM; Supporting Information Table S3).

3.2 | Tissue-specific variations in lipid content and FA composition across salinities and silvering stages

3.2.1 | Muscle

The overall FA composition of eel muscle tissue varied across salinity zones (PERMANOVA, pseudo-$F = 9.6$, $P = 0.0001$) and silvering stages (pseudo-$F = 2.9$, $P = 0.002$). An interaction effect (salinity zone × silvering stage) was also observed (pseudo-$F = 2.2$, $P = 0.007$). The SW and BW eel muscle samples were 91.7% (SIMPER) similar, whereas similarity between BW and FW samples was 89.7%, and that between SW and FW samples was 90.0%. The muscle tissue of SW and BW eels was characterized by higher overall content of 17:1n-7, 20:1n-9 and EPA than in FW eels (ANOVA and ANOVA on ranks, followed by associated *post hoc* tests, all at $P < 0.05$; Table 2 and Supporting Information Table S4). In addition, SW eels had the largest proportions of 18:1n-9. In contrast, FW eels had larger proportions of 14:0, 18:2n-6, 18:3n-3 and ARA. The muscle of FW eels also had a higher average lipid content than that of SW and BW eels, although the difference was significant only with BW eels. Similar results were obtained when considering stage II eels only (all at $P < 0.05$; Supporting Information Table S5), except for the total lipid content in their muscles, which did not vary significantly across salinity zones (despite observing a similar pattern of a higher mean content in FW eels).

Total lipid content increased with stage, along with that of 16:1n-7 and 18:1n-9 (ANOVA and ANOVA on ranks, followed by the associated *post hoc* comparisons, were all at $P < 0.05$; Table 3 and Supporting Information Table S6). In contrast, proportions of 18:0, EPA and DHA decreased in later stage eels ($P < 0.05$; Table 3 and Supporting Information Table S6). Likewise, total lipid content and proportions of 14:0, 16:1n-7, 18:1n-9, 24:1n-9 and 18:2n-6 increased significantly in the muscle of BW eels with advancing stage, while those of 16:0, 18:0, 17:1n-7, EPA and 22:5n-3 diminished

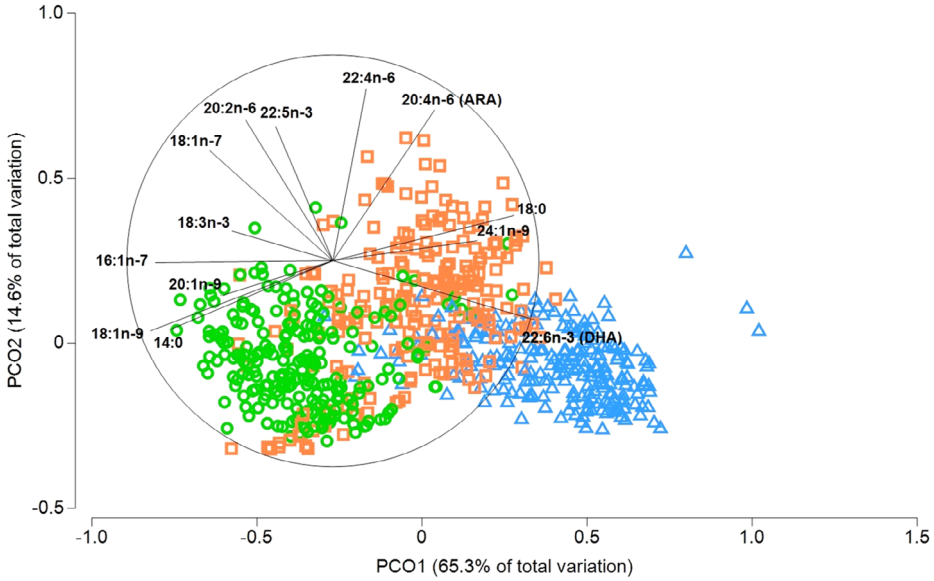

FIGURE 2 Differences in the fatty acid FA composition of European eel *Anguilla anguilla* across eel tissues/organs plotted using principal coordinates analysis (PCoA). Reported in the plot are the FA contributing ≥50% of the variability. The acronyms ARA and DHA indicate the acids arachidonic and docosahexaenoic, respectively. (△) eye; (□) liver; (○) muscle

($P < 0.05$; Supporting Information Table S7). Muscle lipid content was significantly correlated with eel body size (Spearman rank order correlation: $r_s = 0.605$, $n = 233$, $P < 0.001$, total length; $r_s = 0.680$, $n = 233$, $P < 0.001$, wet mass).

3.2.2 | Liver

The FA composition of eel liver varied across salinity zones (PERMANOVA, pseudo-$F = 3.1$, $P = 0.0087$), but not across stages ($P > 0.05$); an interaction effect of salinity zone × stage (PERMANOVA, pseudo-$F = 1.9$, $P = 0.0293$) was also observed. Similarity in the liver FA composition between SW and FW eels was 90.1% (SIMPER), whereas SW and BW samples were 89.9% similar, and BW and FW samples were 89.4% similar. The liver of SW eels had lower proportions of 18:0, 18:2n-6, 18:3n-3, and ARA (ANOVA and ANOVA on ranks, followed by *post hoc* tests, were all at $P < 0.05$) than the liver of BW or FW eels, but had the highest mean EPA proportions (Table 2 and Supporting Information Table S4). The liver of FW eels had a higher mean total lipid content than that of BW or SW eels, but differed significantly only from BW eels. These results were confirmed when the analysis was repeated for stage II eels only. However, as for muscle tissue samples, the lipid content did not vary significantly across salinity zones (all tests at $P > 0.05$; Supporting Information Table S5), despite detecting a similar pattern of mean values.

The proportions of 16:1n-7 and 18:1n-7 increased overall, while EPA decreased with advancing stage (ANOVA and ANOVA on ranks, followed by associated *post hoc* tests, all at $P < 0.05$; Table 4 and Supporting Information Table S6). Similarly, proportions of EPA were lower in the liver of later stage BW eels, together with those of 22:4n-6 and 22:5n-3 ($P < 0.05$; Supporting Information Table S7). The opposite trend was observed for the proportions of 16:0, 20:1n-9, 18:2n-6 and DHA ($P < 0.05$).

3.2.3 | Eyes

The FA composition in eyes varied significantly across salinity zones (PERMANOVA, pseudo-$F = 7.6$, $P = 0.0001$) and stages (PERMANOVA, pseudo-$F = 5.3$, $P = 0.0002$), but there was no significant interaction. The SW and BW eye samples were 91.6% similar (SIMPER), whereas BW and FW samples were 90.5% similar, and SW and FW samples were 90.4% similar. SW eels had the greatest overall proportions of 18:1n-9, 20:1n-9 and EPA, as well as lipid content, in their eyes (ANOVA and ANOVA on ranks, followed by *post hoc* tests, all at $P < 0.05$). The eyes of BW eels had the highest proportions of DHA (Table 2 and Supporting Information S4). In contrast, the eyes of FW eels were characterized by larger proportions of 14:0, 16:1n-7, 17:1n-7, 18:2n-6, 18:3n-3 and ARA than their BW and SW counterparts. These results on FA composition were confirmed when analysing the eyes of only stage II eels (all at $P < 0.05$; Supporting Information Table S5). However, as seen within muscle and liver, total lipid content did not vary across salinity zones, although a similar pattern in mean values was observed.

The proportions of MUFAs (*i.e.*, 16:1n-7, 17:1n-7, 18:1n-9, 18:1n-7, 20:1n-9) in eel eyes increased with advancing stage, along with those of ARA (ANOVA and ANOVA on ranks, followed by associated *post hoc* tests, all at $P < 0.05$; Table 5 and Supporting Information Table S6). In contrast, the relative proportions of 18:0, EPA and DHA were lower in the eyes of later stage eels. Similar results were obtained when analyses were repeated using BW eels only (Supporting Information Table S7).

4 | DISCUSSION

Aquatic environments varying in salinity provide different dietary resources in both quantitative and qualitative terms (*e.g.*, Parzanini *et al.*, 2020). Thus, residing in different salinity habitats may affect

TABLE 2 Mean (± S.D.) total lipid content (% DW) and fatty acid FA composition (%), and sample sizes for muscle, liver and eyes of European eel *Anguilla anguilla* across seawater (SW) brackish water (BW) and freshwater (FW) habitats

	Muscle			Liver			Eye		
	SW	BW	FW	SW	BW	FW	SW	BW	FW
FA	$n = 83$	$n = 120$	$n = 30$	$n = 83$	$n = 120$	$n = 30$	$n = 81$	$n = 116$	$n = 30$
Total lipid	27.5 ± 15.0a	22.4 ± 12.4b	28.7 ± 15.2a	23.0 ± 6.8ab	22.4 ± 6.1a	26.4 ± 8.7b	11.0 ± 6.4a	8.8 ± 2.7b	9.3 ± 3.6ab
Saturated									
14:0	4.3 ± 0.7a	3.9 ± 1.0b	4.8 ± 0.9a	1.8 ± 0.4	1.7 ± 0.5	1.7 ± 0.5	1.8 ± 0.6a	1.6 ± 0.5b	2.1 ± 0.7a
16:0	19.9 ± 1.2	20.3 ± 1.4	20.0 ± 1.4	19.8 ± 2.1ab	20.4 ± 2.8a	18.8 ± 1.7b	18.6 ± 1.6	18.9 ± 1.9	18.6 ± 1.0
18:0	4.2 ± 0.9a	4.7 ± 1.0b	4.3 ± 0.6ab	7.9 ± 1.5a	8.6 ± 1.7b	9.7 ± 1.8c	9.3 ± 1.7a	10.7 ± 1.6b	9.8 ± 1.9a
Monounsaturated									
16:1n-7	7.2 ± 1.6	7.1 ± 2.1	7.6 ± 1.1	4.1 ± 1.2	3.9 ± 1.4	3.6 ± 1.1	3.2 ± 1.2a	2.8 ± 1.1b	3.6 ± 1.4a
17:1n-7	0.6 ± 0.3ab	0.6 ± 0.3a	0.4 ± 0.3b	0.5 ± 0.2a	0.4 ± 0.2b	0.4 ± 0.1ab	0.9 ± 0.4a	1.0 ± 0.4ab	1.2 ± 0.2b
18:1n-9	27.7 ± 5.0a	25.3 ± 4.8b	22.8 ± 2.9c	20.0 ± 6.6	19.8 ± 9.9	18.1 ± 6.8	14.0 ± 4.1a	11.8 ± 3.4b	12.4 ± 3.5ab
18:1n-7	4.9 ± 1.2	4.6 ± 1.3	4.9 ± 0.7	4.5 ± 1.4	4.3 ± 1.4	4.1 ± 0.7	3.2 ± 0.8a	2.8 ± 0.7b	3.1 ± 0.7a
20:1n-9	1.8 ± 1.1a	1.3 ± 0.6a	0.6 ± 0.1b	1.4 ± 0.6a	1.1 ± 0.5b	0.7 ± 0.2c	0.9 ± 0.6a	0.6 ± 0.3b	0.4 ± 0.1c
24:1n-9	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.1	0.6 ± 0.2	0.6 ± 0.2	0.5 ± 0.2	0.7 ± 0.3	0.7 ± 0.3	0.8 ± 0.4
Polyunsaturated									
Omega-6									
18:2n-6	1.2 ± 0.4a	1.5 ± 0.7a	4.3 ± 1.0b	0.8 ± 0.2a	1.1 ± 0.6a	2.0 ± 0.6b	0.5 ± 0.3a	0.6 ± 0.4a	1.8 ± 1.0b
20:2n-6	0.6 ± 0.3	0.7 ± 0.3	0.6 ± 0.2	0.7 ± 0.3	0.8 ± 0.4	0.6 ± 0.2	0.4 ± 0.4	0.3 ± 0.2	0.3 ± 0.1
20:4n-6 (ARA)[a]	2.0 ± 0.9a	2.4 ± 1.7a	4.3 ± 1.1b	5.6 ± 2.0a	5.8 ± 2.5a	9.9 ± 2.3b	4.4 ± 0.9a	4.3 ± 1.2a	7.6 ± 1.0b
22:4n-6	0.9 ± 0.5a	1.1 ± 1.1a	1.0 ± 0.2b	1.8 ± 1.0	2.1 ± 2.0	1.8 ± 0.4	1.3 ± 0.6	1.4 ± 1.0	1.4 ± 0.3
Omega-3									
18:3n-3	1.0 ± 0.4a	1.2 ± 0.5b	3.2 ± 0.7c	0.5 ± 0.2a	0.7 ± 0.4a	1.3 ± 0.5b	0.4 ± 0.3a	0.4 ± 0.3a	1.4 ± 0.9b
20:5n-3 (EPA)[a]	4.8 ± 0.7a	4.9 ± 1.0a	3.4 ± 0.7b	5.4 ± 1.2a	5.3 ± 1.7a	4.5 ± 1.1b	3.6 ± 1.0a	3.1 ± 1.0b	2.7 ± 0.7c
22:5n-3	3.1 ± 0.6	3.2 ± 1.1	3.0 ± 0.5	3.5 ± 1.0	3.5 ± 1.6	3.1 ± 0.6	2.5 ± 0.6a	2.3 ± 0.8b	2.1 ± 0.4b
22:6n-3 (DHA)[a]	10.4 ± 3.9	11.7 ± 5.4	9.6 ± 2.9	16.5 ± 5.4	15.4 ± 5.2	15.7 ± 3.8	30.7 ± 7.7a	33.8 ± 6.4b	27.8 ± 6.7a

Note. A common letter code identifies no significant differences in the pairwise comparisons ($P > 0.05$) for a given tissue/organ type between salinity zones.

[a]ARA, EPA and DHA indicate arachidonic, eicosapentaenoic and docosahexaenoic acid, respectively.

growth, reproduction and survival of *A. anguilla*. We sought to understand the benefits, if any, for *A. anguilla* to live in any given habitat, or mix of habitats, during their growth phase by testing whether total lipid content and FA composition of various eel tissues and organs differed based on the salinity where they were captured, as well as their silvering stage.

FW and SW eels had an overall higher lipid content in their tissues compared to BW eels, but these differences were not significant when only stage II eels were considered (although similar patterns across salinity zones were observed). This highlights the importance of accounting for eel silvering stage when comparing eels from different salinity zones/habitats, and sampling equally (as much as feasible) within each stage to avoid sampling bias. At the same time, FW and SW eels had the most divergent FA profiles (specifically in their muscle and eyes), while the FA profiles of BW eels were more similar to those of SW eels. The result may be due to similarities in diet between SW and BW eels or for other reasons as discussed below. Regardless, the FA profiles of eels mirrored those of their feeding habitats, as expected. Furthermore, as hypothesized, the proportions of MUFAs (*e.g.*, 16:1n-7 palmitoleic acid and 18:1n-9 oleic acid) increased overall with advancing stage in all of the tissues and organs analysed. In contrast, those of certain SFAs (*e.g.*, 18:0, stearic acid) and PUFAs (*e.g.*, EPA and DHA) decreased. Similar results were obtained for analyses with only BW eels, suggesting that eels undergo the same biochemical/physiological growth processes regardless of the habitat (SW, BW or FW) in which they reside. However, contrary to initial expectations, the DHA content decreased with advancing stage.

Previous studies have noted intraspecific variability in the biochemical composition of *A. anguilla* across salinities (*e.g.*, in the stable isotopes composition of muscle, Harrod *et al.*, 2005; and FA, Prigge *et al.*, 2012; Ghazali *et al.*, 2013; Vasconi *et al.*, 2019), as well as across life history stages (*e.g.*, in lipid metabolism and blood components, Larsson & Fange, 1969). Here we considered both sources of variation (and their interaction) when analysing eel lipid profiles across three

TABLE 3 Mean (± S.D.) total lipid content (% DW) and fatty acid FA composition (%), and sample sizes for muscle samples of European eel *Anguilla anguilla* across silvering stages (*i.e.*, I, II, III, IV, V)

	Muscle				
	I	II	III	IV	V
FA	*n* = 38	*n* = 156	*n* = 36	*n* = 1	*n* = 2
Total lipids	12.7 ± 6.8a	26.1 ± 12.1b	31.9 ± 17.8b	16.2	56.2 ± 11.3
Saturated					
14:0	3.5 ± 0.9a	4.3 ± 0.8b	4.1 ± 1.2b	3.9	5.3 ± 0.0
16:0	20.1 ± 1.1	20.2 ± 1.4	19.9 ± 1.4	20.0	19.4 ± 1.7
18:0	5.4 ± 0.9a	4.3 ± 0.7b	4.3 ± 1.3b	4.7	3.2 ± 0.4
Monounsaturated					
16:1n-7	5.7 ± 1.3a	7.5 ± 1.6b	7.6 ± 2.2b	7.6	9.1 ± 1.1
17:1n-7	0.6 ± 0.3a	0.6 ± 0.3ab	0.5 ± 0.4b	0.0	0.6 ± 0.1
18:1n-9	21.9 ± 4.9a	26.3 ± 4.3b	27.6 ± 5.8b	29.5	26.0 ± 0.3
18:1n-7	4.4 ± 1.1	4.8 ± 1.3	5.0 ± 1.2	5.8	4.6 ± 0.7
20:1n-9	1.2 ± 0.6	1.4 ± 0.9	1.5 ± 1.0	1.0	2.7 ± 0.9
24:1n-9	0.3 ± 0.1a	0.2 ± 0.1b	0.2 ± 0.2b	0.2	0.2 ± 0.1
Polyunsaturated					
Omega-6					
18:2n-6	1.5 ± 1.0	1.8 ± 1.2	1.9 ± 1.0	4.1	1.3 ± 1.0
20:2n-6	0.7 ± 0.4	0.6 ± 0.3	0.6 ± 0.3	0.8	0.4 ± 0.1
20:4n-6 (ARA)[a]	2.9 ± 1.7	2.3 ± 1.3	2.9 ± 2.1	4.6	1.5 ± 0.2
22:4n-6	1.3 ± 1.3	0.9 ± 0.8	0.9 ± 0.5	1.3	0.4 ± 0.1
Omega-3					
18:3n-3	1.4 ± 0.6a	1.5 ± 0.9a	1.1 ± 0.8b	2.0	2.4 ± 0.8
20:5n-3 (EPA)[a]	5.3 ± 1.1a	4.6 ± 0.9b	4.1 ± 0.8c	1.5	4.5 ± 0.1
22:5n-3	3.6 ± 1.5	3.0 ± 0.7	3.0 ± 0.7	2.1	2.6 ± 0.3
22:6n-3 (DHA)[a]	14.9 ± 4.7a	10.4 ± 4.2b	9.8 ± 5.0b	6.7	8.5 ± 2.1

Note. A common letter code identifies no significant differences in the pairwise comparisons ($P > 0.05$) for eel muscle tissue between stages.

[a]ARA, EPA, and DHA indicate arachidonic, eicosapentaenoic, and docosahexaenoic acid, respectively.

main tissues/organs. Thus, this investigation provides a more comprehensive assessment of the respective contributions of salinity and lifecycle stage. Since the FA composition of *A. anguilla* has already been described in detail (*e.g.*, Lie *et al.*, 1990), and our results are consistent with earlier reports, we here focus on the physiological, biological and ecological implications related to the influences of salinity and lifecycle stage, aspects not previously considered in this context.

4.1 | Variations in lipid content and FA composition across salinities

FW eels, followed by SW eels, had the highest overall lipid content in their muscle and liver tissues. Conversely, SW eels had the greatest lipid content in their eyes and BW eels exhibited the lowest values in all three tissues. As noted above, the differences in lipid content were not significant when analyses were repeated for stage II eels only. Stage II eels are still yellow eels ('growth phase'; Durif *et al.*, 2005) and hence may have only recently, or not yet, started to accumulate fat reserves. This putative 'low demand' for fat reserves suggests that these stage II eels are potentially able to fulfil their energy needs irrespective of the habitat (SW, BW and/or FW) in which they reside.

Intraspecific variation in lipid content and composition of fish across salinity zones may be related to diet (*e.g.*, Vasconi *et al.*, 2019). Osmoregulatory and other physical adjustments due to variations in environmental salinity may also modulate lipid metabolism, as has been reported in several species, including guppy *Poecilia reticulata* Peters 1859 (Daikoku *et al.*, 1982), European bass *Dicentrarchus labrax* (L. 1758) (Roche *et al.*, 1983), mullet *Mugil cephalus* Linnaeus 1758 (Khériji *et al.*, 2003) and *A. anguilla* (Ghazali *et al.*, 2013). For instance, Daikoku *et al.* (1982) and Roche *et al.* (1983) noted reductions in total lipid content in the muscle of fish exposed to salinity variations. Similarly, Ghazali *et al.* (2013) observed that the lipid content in the muscle of FW yellow eels decreased after they fasted in SW for 4 weeks. The authors suggested that these eels most likely used the energy stored in the muscle lipids to fuel their metabolic needs while adjusting to salinity variations (Ghazali *et al.*, 2013).

TABLE 4 Mean (± S.D.) total lipid content (% DW) and fatty acid FA composition (%), and sample sizes for liver samples of European eel *Anguilla anguilla* across silvering stages (*i.e.*, I, II, III, IV, V)

	Liver				
	I	II	III	IV	V
FA	$n = 38$	$n = 156$	$n = 36$	$n = 1$	$n = 2$
Total lipids	33.7 ± 77.4	23.3 ± 6.3	24.0 ± 8.9	39.9	24.6 ± 11.6
Saturated					
14:0	1.6 ± 0.4	1.7 ± 0.5	1.8 ± 0.5	2.4	2.4 ± 0.7
16:0	20.1 ± 2	19.7 ± 2.5	20.8 ± 3.1	19.1	21.8 ± 0.5
18:0	9.1 ± 1.5a	8.4 ± 1.7b	8.2 ± 1.9b	7.0	6.8 ± 0.4
Monounsaturated					
16:1n-7	3.3 ± 1.1a	3.9 ± 1.2b	4.6 ± 1.4c	5.1	5.2 ± 2.0
17:1n-7	0.4 ± 0.1	0.4 ± 0.2	0.5 ± 0.1	0.4	0.4 ± 0.1
18:1n-9	17.3 ± 8.5a	19.9 ± 8.6b	20.5 ± 7.7b	32.9	22.6 ± 2.8
18:1n-7	3.9 ± 1.3a	4.3 ± 1.4a	4.9 ± 1.2b	4.5	3.6 ± 1.2
20:1n-9	1.0 ± 0.6	1.2 ± 0.6	1.2 ± 0.4	1.1	2.0 ± 0.1
24:1n-9	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.5	0.5 ± 0.3
Polyunsaturated					
Omega-6					
18:2n-6	1.0 ± 0.5	1.0 ± 0.6	1.3 ± 0.7	2.1	1.0 ± 0.9
20:2n-6	0.8 ± 0.4	0.7 ± 0.4	0.7 ± 0.2	0.8	0.4 ± 0.2
20:4n-6 (ARA)[a]	6.4 ± 2.6	6.2 ± 2.7	6.5 ± 2.8	6.9	3.5 ± 0.9
22:4n-6	2.5 ± 2.2	1.9 ± 1.5	1.8 ± 0.8	2.2	0.5 ± 0.0
Omega-3					
18:3n-3	0.7 ± 0.4	0.7 ± 0.4	0.6 ± 0.3	0.9	1.1 ± 0.1
20:5n-3 (EPA)[a]	6.0 ± 1.5a	5.3 ± 1.4b	4.2 ± 1.2c	1.4	5.0 ± 2.4
22:5n-3	3.7 ± 1.6	3.4 ± 1.3	3.3 ± 1.2	2.2	2.5 ± 0.4
22:6n-3 (DHA)[a]	16.9 ± 5.7	16.0 ± 5.2	14.2 ± 3.8	7.1	16.1 ± 5.1

Note. A common letter code identifies no significant differences in the pairwise comparisons ($P > 0.05$) for eel liver between stages.

[a]ARA, EPA and DHA indicate arachidonic, eicosapentaenoic and docosahexaenoic acid, respectively.

Coupled with the findings above, we propose that the variations in total lipid content across SW, BW and FW eels observed in this study reflect the differences in life history strategies and migratory behaviours of eels sampled across salinities. Specifically, BW eels showed the lowest lipid content, suggesting that these eels (a) may eat less food compared to their SW and FW counterparts, and (b) may undertake frequent, energy-consuming, interhabitat migrations (habitat shifting) that prevent them from accumulating large amounts of energy reserves. Alternatively, these eels (c) may experience variable salinities in an estuary or salinity mixing zone (without true inter-habitat shifting movements). The relatively lower amount of lipids in BW eels may result from the continuous changes in osmo- and ionoregulation processes that consume metabolic energy in response to changes in salinity. In contrast, the SW and FW eels that had the highest lipid content (in muscle and liver) may occupy the same habitats for a longer period (*i.e.*, SW and FW residency), allowing them to lower metabolic costs and accumulate greater energy reserves in their tissues.

Significant differences across salinity zones were observed when analysing the composition of individual FAs. In particular, the muscle of SW eels was characterized by the greatest proportions of 18:1n-9, 20:1n-9 (gondoic acid), EPA and DHA. In contrast, their FW counterparts had higher proportions of 14:0 (myristic acid), 18:2n-6 (linoleic acid), 18:3n-3 (α-linolenic acid) and ARA. Given that the FA composition of a consumer typically reflects that of its diet (Dalsgaard *et al*., 2003; Napolitano, 1999), especially for white muscle tissue that serves as the main fat depot for most fishes (Tocher, 2003), the observed patterns most likely reflect eel dietary differences across salinity zones. Our results for muscle tissue are consistent with those reported by Prigge *et al*. (2012), who noted that *A. anguilla* fed SW versus FW diets had different FA compositions, with FW diet eels having lower EPA/ARA and n-3/n-6 ratios in their muscle than the SW diet eels.

Organisms residing in FW habitats are typically richer in n-6 PUFAs (including 18:2n-6 and ARA). However, organisms living in SW habitats have higher proportions of n-3 PUFAs (such as EPA and DHA), mostly due to the large production of these FA by the abundant diatoms and dinoflagellates in the temperate marine regions in which these eels reside (Dalsgaard *et al*., 2003; Parzanini *et al*., 2020;

TABLE 5 Mean (± S.D.) total lipid content (% DW) and fatty acid FA composition (%), and sample sizes for eye samples of European eel *Anguilla anguilla* across silvering stages (*i.e.*, I, II, III, IV, V)

	Eye				
	I	II	III	IV	V
FA	$n = 37$	$n = 152$	$n = 35$	$n = 1$	$n = 2$
Total lipids	9.9 ± 3.1	10.0 ± 9.7	10.1 ± 3.7	5.1	20.8 ± 0.2
Saturated					
14:0	1.5 ± 0.4	1.8 ± 0.6	1.7 ± 0.6	2.4	3.7 ± 0.2
16:0	18.4 ± 1.9	18.8 ± 1.7	18.8 ± 1.6	19.0	19.2 ± 0.8
18:0	11.1 ± 1.1a	10.0 ± 1.8b	9.5 ± 1.4b	7.8	4.7 ± 0.5
Monounsaturated					
16:1n-7	2.4 ± 0.5a	3.1 ± 1.2b	3.3 ± 1.1b	4.9	7.1 ± 0.3
17:1n-7	1.0 ± 0.3ab	1.0 ± 0.4a	0.8 ± 0.4b	1.2	0.8 ± 0.1
18:1n-9	10.6 ± 2.3a	12.7 ± 3.9b	13.7 ± 3.1b	19.4	22.3 ± 0.3
18:1n-7	2.6 ± 0.5a	3.0 ± 0.8b	3.2 ± 0.8b	4.5	4.6 ± 0.2
20:1n-9	0.6 ± 0.3a	0.7 ± 0.5ab	0.7 ± 0.2b	0.7	1.8 ± 0.2
24:1n-9	0.7 ± 0.3	0.7 ± 0.3	0.8 ± 0.4	1.0	0.4 ± 0.2
Polyunsaturated					
Omega-6					
18:2n-6	0.5 ± 0.4	0.7 ± 0.6	0.8 ± 0.7	2.7	1.4 ± 1.2
20:2n-6	0.4 ± 0.6	0.3 ± 0.2	0.3 ± 0.1	0.6	0.3 ± 0.1
20:4n-6 (ARA)[a]	4.5 ± 1.6a	4.7 ± 1.5ab	5.2 ± 1.3b	9.1	2.9 ± 0.2
22:4n-6	1.3 ± 0.9	1.3 ± 0.7	1.7 ± 0.9	2.3	0.6 ± 0.1
Omega-3					
18:3n-3	0.5 ± 0.3a	0.6 ± 0.5a	0.4 ± 0.5b	1.4	1.8 ± 0.2
20:5n-3 (EPA)[a]	3.2 ± 0.9a	3.3 ± 1.0b	2.9 ± 1.0ab	1.7	5.0 ± 0.3
22:5n-3	2.3 ± 0.8	2.3 ± 0.6	2.3 ± 0.7	1.8	2.5 ± 0.2
22:6n-3 (DHA)[a]	35.1 ± 5.8a	31.7 ± 7.2b	31.1 ± 6.7b	16.6	15.7 ± 0.7

Note. A common letter code identifies no significant differences in the pairwise comparisons ($P > 0.05$) for eel eyes between stages.

[a]ARA, EPA and DHA indicate arachidonic, eicosapentaenoic and docosahexaenoic acid, respectively.

Steffens, 1997). The high proportion of 20:1n-9 observed in SW eels is also reflective of their feeding habitat as FAs of the series 20:1 and 22:1 are commonly found in SW herbivorous zooplankton, including Arctic calanoid copepods (Falk-Petersen *et al*., 2000). Similarly, wild eels collected from a BW lagoon had higher ARA and n-6 PUFA proportions than wild SW eels (Vasconi *et al*., 2019). The diet of *A. anguilla* can vary significantly due to its highly opportunistic feeding behaviour (Bouchereau *et al*., 2009), even when individuals are collected from nearby sites, with differences in feeding behaviours expected to be reflected in eel FA composition.

It is more difficult to link the FA composition of the liver and eyes to dietary sources. This is due to the highly specific functional role of these organs, which largely dictates their biochemical composition (*e.g*., lipid class and FA composition) and properties. While eels store fat in both muscle and liver (Burgos *et al*., 1989; Otwell & Rickards, 1981), liver is also the main site of lipid metabolism and energy mobilization (Hansen & Abraham, 1983; Tocher, 2003). As liver is characterized by higher biochemical turnover rates than white muscle, it is typically the less preferred tissue choice in long-term dietary studies (Parzanini *et al*., 2019). For the eyes, contradictory results have been reported regarding diet effects on FA composition (*e.g*., Brodtkorb *et al*., 1997; Li *et al*., 2014; Navarro *et al*., 1997). Here, patterns of FA composition in muscle, liver and eye were broadly comparable in terms of differences across salinity zones, suggesting that diet affects the FA composition of eye and liver as well as muscle tissue. Similarly, SW rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) had higher n-3/n-6 ratios in their livers than their FW equivalents (Haliloğlu *et al*., 2004). In addition to the influence of feeding habitat/diet, we cannot exclude the possibility that these tissues, especially liver due to its central role in lipid metabolism (Hansen & Abraham, 1983), may undergo biochemical and physiological changes in response to changing salinity and other environmental conditions (*e.g*., temperature) across FW, BW and SW habitats. This has been observed in *A. anguilla* (Prigge *et al*., 2012) as well as other fish species, including red drum *Sciaenops ocellatus* (L. 1766) (Craig *et al*., 1995), Arctic charr *Salvelinus alpinus* (L. 1758) (Bystriansky *et al*., 2007) and Mexican silverside *Chirostoma estor* Jordan 1880 (Fonseca-Madrigal *et al*., 2012).

4.2 | Variations in lipid content and FA composition along with silvering stage

Proportions of 16:1n-7, 17:1n-7 (heptadecenoic acid), 18:1n-9, 18:1n-7 (vaccenic acid), 20:1n-9 and ARA increased in eel eyes with advancing stage. In contrast, 18:0, EPA and DHA decreased in the eyes of later stages, whereas no change was detected in total lipid content. While a previous study by Kirsche (1966) suggested that yellow eels make little or no use of their eyes, those of silver eels are much larger, including the retinal surface, with structural changes (*e.g.*, in the type, amount and distribution of photoreceptors and visual pigments) occurring in the early phase of sexual maturation (Beullens *et al.*, 1997; Pankhurst, 1982; Tesch, 1977). These modifications are thought to ultimately increase sensitivity, which is critical for the low-light conditions that eels would encounter during their deep-sea migration (Rousseau *et al.*, 2012; Tesch, 1977). The growing MUFA proportions with advancing stage observed in this study might be due to the overall increase in eye and eel size (confirmed by the exponential relationship between eye diameters and body length; Pankhurst, 1982). Increasing proportions of ARA may be related to its role as a precursor of important eicosanoids and signalling molecules, such as cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) (Estevez & Kanazawa, 1996). In vertebrate eyes, cAMP and cGMP both control phototransduction and photoreceptor sensitivity (Astakhova *et al.*, 2012; Estevez & Kanazawa, 1996). Therefore, eels may acquire greater light sensitivity throughout development by increasing the relative proportion of ARA in their eyes.

The proportion of DHA decreased with advancing stage, although it remained a dominant component in the FA composition of the eyes. DHA is a main FA found in fish retina (Mourente, 2003), being particularly abundant in rod outer segment membranes, and is thought to play a major role in fish vision (Bell *et al.*, 1995). For instance, deficiency of dietary DHA altered the vision of juvenile *C. harengus* at low-light intensities (Bell *et al.*, 1995). Our result may relate to the fact that whole eyes were analysed, rather than retinal tissue alone, thus masking the actual changes in the FA composition of the retina. Regardless, the literature indicates that all changes occurring in the eyes of anguillid eels during maturation are preparatory for the migration to the spawning grounds (Durif *et al.*, 2006; Pankhurst, 1982; Rousseau *et al.*, 2012).

Similarly, in muscle and liver, the proportions of MUFAs (*e.g.*, 16:1n-7 and 18:1n-9) increased more than those of SFA and PUFA along with stage. This result may be related to overall lipid accumulation prior to migration, suggesting that eels preferentially utilize MUFAs for energy production. Eels require sufficient lipid stores (*i.e.*, >20% muscle fat content) for migration and spawning success (Boëtius & Boëtius, 1980; Larsson *et al.*, 1990; Svedäng & Wickström, 1997). It is also critical that these energy reserves are accumulated prior to migration, as the gut degenerates at the start of migration (Durif *et al.*, 2005; Tesch, 1977). In support of this interpretation, total lipid content in muscle tissue also increased with advancing stage (especially between stages I and II, and between stages I and III). Furthermore, although saturated FAs (SFAs) are generally better suited for energy production due to their structure (Spielmann *et al.*, 1988), MUFAs (including 18:1n-9, 20:1n-9 and 22:1n-11 docosenoic acid) represent a main source of energy in fish. This is because MUFAs are just as likely to be readily oxidized (Tocher, 2003), but possess greater caloric content compared to SFA of the same chain length (Freedman & Bagby, 1989). Hence, MUFAs may provide critical metabolic energy to migratory eels. For instance, 18:1n-9 was abundant (>12%) in all tissue/organ types studied here and was found to increase significantly with advancing stage. Notably, 18:1n-9 is a major energy source fuelling fish spawning migrations (Penney & Moffitt, 2015; Wiegand & Idler, 1985).

The relative overall decrease in PUFAs in later stage eels may be due to the fact that these FAs, especially n-3 LC-PUFAs (*e.g.*, EPA, DHA) and n-6 LC-PUFAs (*e.g.*, ARA), are mobilized from the muscle and directed through the liver towards gonad and egg development (Ozaki *et al.*, 2008). These essential FAs promote oocyte growth (da Silva *et al.*, 2016) in anguillid eels, as well as reproductive success and broodstock growth in the context of aquaculture (Furuita *et al.*, 2007). Furthermore, a diet devoid of optimal DHA/EPA/ARA ratios has been suggested to alter gamete production and quality in *A. anguilla* (Baeza *et al.*, 2014; Butts *et al.*, 2015) and to cause ovulation issues in cultured *A. japonica* (Higuchi *et al.*, 2019). We observed decreases in EPA proportions in liver and muscle tissue with advancing stage, while those of DHA decreased only in muscle tissues and those of ARA were conserved across stages I, II and III. The low sample size representing later stages IV ($n = 1$) and V ($n = 2$) prevented a full assessment of differences in the FA composition until the migratory stages IV and V. Nonetheless, our results suggest that EPA is among the first critical molecules to be mobilized in eels for sexual maturation. Liver EPA, for instance, is a key FA during the spermiation process in laboratory-reared males of *A. anguilla* (Baeza *et al.*, 2014).

4.3 | Conclusion and practical applications

Clear patterns were detected in total lipid content and FA composition of juveniles of *A. anguilla* sampled across salinities and silvering stages during the continental phase of their life history. The observed FA trends across salinity zones likely reflect underlying differences in diet (*i.e.*, SW *vs.* FW feeding habitat), life history strategy (*i.e.*, SW or FW residents *vs.* habitat shifters) and adaptive responses to the diversified environmental conditions characterizing SW *versus* BW *versus* FW habitats. The observed patterns associated with advancing stage are also likely indicative of the biological changes occurring in preparation for the final stage of puberty (*i.e.*, silvering) leading to the seaward spawning migration. Future studies, including manipulative laboratory experiments, should further investigate the combined effects of salinity and silvering stage on lipid metabolism.

Although widespread across Europe, all *A. anguilla* individuals spawn in the Sargasso Sea (Als *et al.*, 2011; Schmidt, 1923). The quality of eels varies across their geographical distribution, partly due to their contaminant and parasite loads, but also to their lipid levels (ICES, 2013). As *A. anguilla* is critically endangered, baseline

information on their FA profiles throughout their lifecycle and habitat(s) in which they live and grow is needed to support conservation initiatives for the species, with the end goal being to potentially prioritize/protect certain areas/habitats and/or facilitate passage between habitats. For instance, eels from FW habitats had the highest overall lipid content in their muscle and liver tissues. Although many eels in the northern part of the geographical distribution skip the FW phase, there is an advantage to this life history strategy from a lipid perspective. Our results suggest that BW eels spend a lot of energy adapting to a changing salinity environment. Even though shifting habitats may increase food options, decrease risk of predation and reduce exposure to pathogenic FW parasites, the tactic may not increase their overall fitness and survival, as suggested by Edeline (2007). For these and other reasons, it may be advisable to facilitate/enhance upstream and downstream movements by limiting or removing migratory barriers along the way and/or deploying fish passages (*e.g.*, fish ladders).

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AUTHOR CONTRIBUTIONS

C.M.F.D., H.I.B. and M.R. contributed to the design and implementation of the study, whereas C.P. and M.T.A. contributed to the paper conceptualization. C.P. was also responsible for data curation and analysis, and writing the original draft the manuscript. All the authors reviewed and edited the draft. C.M.F.D., H.I.B. and M.T.A. provided funds and resources for this project.

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