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The Lipid Composition and Biochemistry of the Migrating European Eel (*Anguilla anguilla* L.): A LCMS-Study Following a lipidomics Based Systems Biology Approach

Vincent van Ginneken[1*], Maarten Hekman[2], Elwin Verheij[2]

[1]Bluegreen Technologies, Ginkelseweg, Heelsum, Netherlands

[2]TNO Healthy Living, Zeist, Netherlands

[*]**Corresponding author:** Vincent van Ginneken, Bluegreen Technologies, Ginkelseweg 2, 6866 DZ Heelsum, Netherlands. Email: vvanginneken@hotmail.com

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Abstract

In this study we described the lipid composition of pre-migrants (yellow) and migrants (silver) of European eel -all females- in muscle- and gonad- tissue using LCMS techniques for Cholesteryl Esters (ChE), Lysophosphatidylcholines (LPC), Phosphatidylcholine (PC), Sphingomyelin (SPM), Diacylglycerol (DG) and Triacylglycerol's (TG). Muscle tissue is extremely rich in TGs with the important observation that there was no significant difference between pre-migrants and migrants ($P \le 0.988$). MRI T2-weighted images indicated that the White Adipose Tissue (WAT) stores surround the muscle myotome. In gonad tissue both TGs and ChE are the most abundant lipids. Major observation was that only the Phosphatidylcholine content of the gonad of the migratory animals was for the total amount significantly higher ($P \le 0.0015$) in comparison to pre-migratory stages. Based on product-precursor ratios we calculated following a Systems Biology approach muscle- and gonad- enzymatic activities. Desaturase (C16:1/C16:0), Δ6-desaturase (C18:3/C18:2), elongase (C22:5/C20:5), [elongase+Δ6-desaturase+β-oxidation] (C22:6/C22:5) and lineoyl CoA-desaturase (C20:4/C18:2) could be calculated. For lineoyl CoA-desaturase for the gonad between pre-migrants 1.543±0.2506 and migrants 0.954±0.2033 the enzymatic activity decreased significantly ($P \le 0.00132^{**}$) which is indicative for a 1.7 fold decrease of this enzyme in the gonad of the silver. An important observation was that European eel shows the characteristics of freshwater fish and are able to convert dietary C18 precursor fatty acids to Arachidonic Acid (AA), Eicosapentaenoic Acid (EPA), and Docosahexaenoic Acid (DHA). Specific biomarkers for the different lipid fractions were using a 200% ration for the fraction [migrant]/[pre-migrant]*100%: Ovary PCs: C30-1 208.9%; C32-2 331.81; C34-2 275.0% ; C34-3 338.2%; C34-5 230.0%; C36-2 241.8%; C36-4 215.0%; C36-5 211.2%; C36.6% 280.3%; C38-6 203.4%; C38-7 272.2%; C40-7 214.3%; Muscle TGs: none; Ovary PCs: C34-0 215.9%; ChE C18-3 223.7%; Ovary TGs: C46-4 232.37%; C46-5 308.0%; C48-6 266.3%; C52-9 258.2%. This approach enables us to understand the lipid biochemistry of the endangered European eel prior to its 5,500 km migration to the Sargasso Sea following this lipidomics Systems Biology approach. Most important observation was that there were no significant differences in concentrations for TGs between pre-migrants and migrants ($P \le 0.988$) so we conclude -supported by our MRI measurements- eel fat stores are sufficient to reach the Sargasso Sea.

Keywords: Eel, *Anguilla anguilla*; LCMS, Lipidomics; Systems Biology; Premigrant; Migrant; Yellow; Silver Biomarker; Lipids; Migration; Sargasso Sea.

Introduction

During its life cycle the European eel (*Anguilla anguilla* L.) experiences two periods of metamorphosis. The first transformation is from the planktonic marine stage (*Leptocephalus* larvae) into the glass eel. This occurs during its oceanic migration from the supposed spawning grounds in the Sargasso Sea to the coasts of Europe before entering fresh water [1]. The second (partial) metamorphosis occurs after the juvenile growth and differentiation phase (> 4 years for males, >7 years for females) in the inland waters. The eels transform from yellow eel into silver eel.

During the latter transformation there is some proliferation of the gonads and an increase in eye size. Furthermore, the body color becomes silvery, the alimentary tract shows regression, and the animal becomes fatter [2,3]. The transformation of yellow eel into silver eel is called 'Silvering'and takes place prior to migration. We used Principal Component Analysis (PCA) to characterize the morphological, physiological [3] and endocrine changes [4] that accompany silvering in the European eel (*Anguilla anguilla* L.). Silvering is positively related to external parameters such as eye size, internal maturation parameters like GSI, Vitellogenine (VIT), and blood-substrates such as phospholipids, Free Fatty Acids (FFA), and cholesterol. The Hepatosomatic Index was not significantly different between yellow and silver groups [3]. In contrast, a significant difference was observed for parameters of body constitution (fat, protein, dry matter) between yellow and silver stages [3]. Furthermore, the process of silvering is accompanied with increased levels of cortisol in autumn, which plays a role in mobilization of metabolic energy from body stores towards migratory activity and gonadal growth. So, in previous studies we described hormonal profiles of European eel (*Anguilla anguilla* L.) during silvering. This transformation occurs in association with hormonal surges of Testosterone (T) and Estradiol (E2) but not with Thyroid Hormones (TH) and Growth Hormone (GH) which have a maximum activity in spring and a minimum activity in summer and autumn. It is therefore suggested THs and GH are not important for eel gonadal development in the autumn [4]. Based on PCA analysis with physiological, morphological and endocrine parameters it is concluded that the transition is gradual and that eels go through several stages [5,6]. Based on PCA with physiological, morphological and endocrine parameters, it is concluded that during the process of silvering, several developmental stages can be recognized, with a timeframe of the pre-migratory sedentary yellow phase from April until July, August is a cross-over month, and the migratory silver phase is found from September until November [3]. It is assumed that these changes are part of silvering processes, precedes the spawning migration to the Sargasso, 5,500 km away from Europe [1,2,7]. Silvering of eel is typically related to migration, actually animals remain in a pre-pubertal stage, even at the moment when they leave the coast [5,6]. The mechanisms involved in the onset of silvering are largely unknown. They are important however to understand because eel species around the world are an endangered species in the category **CR** (≈critically endangered; Figure 0). Our research showed that various factors may have contributed to the dwindling of the European eel population such as a). viruses [8,9]; b). swimbladder parasite [10]; c). contamination with PCBs [11,12]; and possibly an interaction with global warming [13] although the latter is solely a hypothesis.

Figure 0: European eel conservation status by IUCN Red List category. The European eel is designated by the IUCN commission as endangered species in the very serious category "Critically Endangered" (CR) [14].

It is generally assumed that silvering is an adaptation of the eel for its 6000-km migration journey. Since the swim-effort is a prerequisite for spawning, it is hypothesized that morphological and physiological changes are more related to swim-endurance than to maturation. It is more advantageous to the animal that maturation is postponed or slowed down till the end of the journey. The increase of metabolic rate and the need for fuel mobilization might thus be more relevant during silvering than the increase of the GSI. Consequently, changes in fat mobilization and thyroxin level [4,15] might be expected. In this study characteristics for 'silvering' in combination with gonad development were described in order to investigate if the process of 'silvering' is accompanied with a development of the gonad. However, studies related to the lipid profile in pre-migrants and migrants have to our awareness solely been performed with the Japanese eel (*Anguilla japonica*) [16] and the shortfinned Australian eel (*Anguilla australis*) [17]. We hypothesize that for the European eel (*Anguilla anguilla*) also in the lipid profiles between the (pre) migrants and migrants differences can be observed in lipid composition of muscle and gonads because the migrants have to perform a tremendous Trans-Atlantic swimming effort of around 5,500 km to the Sargasso Sea [1,2,7] without feeding while the gonads will mature. This study gives with state of the art techniques like LCMS of muscle and gonad of pre-migratory and migratory animals an impression of these lipid changes at the molecular level. In addition, T2-weighted unique MRI images show how the fat depots of triacylglycerols are located along the muscle myotomes.

Material & Methods

Animals

In autumn an experienced fisherman caught in the region South-Holland (vicinity Leiden) during seaward migration randomly eels. Solely females > 200 g were taken in the sample because males in the Netherlands of the European eel have a smaller size. Next, the eels were subdivided in a selective way into pre-migratory and migratory following the next mentioned criteria. The criteria for non-migratory where: lateral side yellow-green shine, no sharp transition from dorsal to ventral side, soft skin.

The criteria for migratory where: silvery shine, sharp transition to a white abdomen, tough skin and enlarged pectoral fins and eyes. In this way we could clearly distinguish two separate groups consisting of 7 migrants (silver) and 6 pre-migrants (yellow) animals. The photo in Figure 1 is a clear reflection of this visual separation between animals.

Figure 1: Top photo of a yellow (non-migrant) eel with at the bottom silver (migrant) stage. Note enlarged eyes of the migrant enlarged pectoral fin and white coloring of the belly (silver=migratory stage). The transformation of yellow to silver eel is characterized by a proliferation of the gonads, an increase in eye size, the body color becomes silvery and the alimentary tract shows a regression. It is assumed that these changes are adaptations for the return journey to the spawning grounds. Little is known about the physiological and endocrine changes during the yellow-silver transition. It was the intention of this study to investigate the process of early maturation during this life stage of the animal following a Systems Biology lipidomics based approach.

Tissues

A muscle or gonad homogenate (~10% wet weight/ vol) in PBS (Phosphate-Buffered Saline) was made by stirring the tissue in a closed tube with small glass beads.

Mass Spectrometry (LC-MS)

As described earlier [18-20], fifty µl of the well mixed tissue homogenate was mixed with 1000 µl IPA containing 4 internal standards. In addition, blood plasma samples of 10 µl plasma were extracted with 300 µl of Isopropanol (IPA) containing several internal standards (IS: C17:0 lysophosphatidylcholine, di-C12:0 phosphatidylcholine, tri-C17:0 glycerol ester, C17:0 cholesteryl ester and heptadecanoic acid (C17:0)). Samples were placed in an ultrasonic bath for 5 minutes. After mixing and centrifugation (10000 rpm for 3 minutes) the supernatant was transferred to an autosampler vial. Thereaftere10 µl of the sample was injected on the LC-MS Instrument (Thermo Electron, San Jose, USA). A Thermo LTQ is a linear ion-trap LC-MS instrument (Thermo Electron, San Jose, USA). Lipids were separated on a 150 x 32 mm id C4 Prosphere column (Alltech, USA) using a methanol gradient in 5 mM ammonium acetate and 0.1% formic acid (mobile phase A: 5% methanol, mobile phase B: 90% methanol). The flowrate was 0.4 ml/min and the gradient was as follows: 0-2 min - 20%B, 2-3 min - 20% to 80%B, 3-15 min - 80% to 100%B, 15-25 min - hold 100%B, 25-32 min -condition at 20% B. The instrument used was a Thermo LTQ equipped with a Thermo Surveyor HPLC pump Data were acquired by scanning the instrument from m/z 300 to 1200 at a scan rate of approximately 2 scans/s in positive ion ESI mode.

Estimation of Enzyme Activity of Desaturases and Elongases in Muscle or Gonad

In addition, elongase desaturase series, from which enzym activities can be calculated based on product-to-precursor ratios of individual measurement of fatty acids [18-20], are depicted in Figure 2.

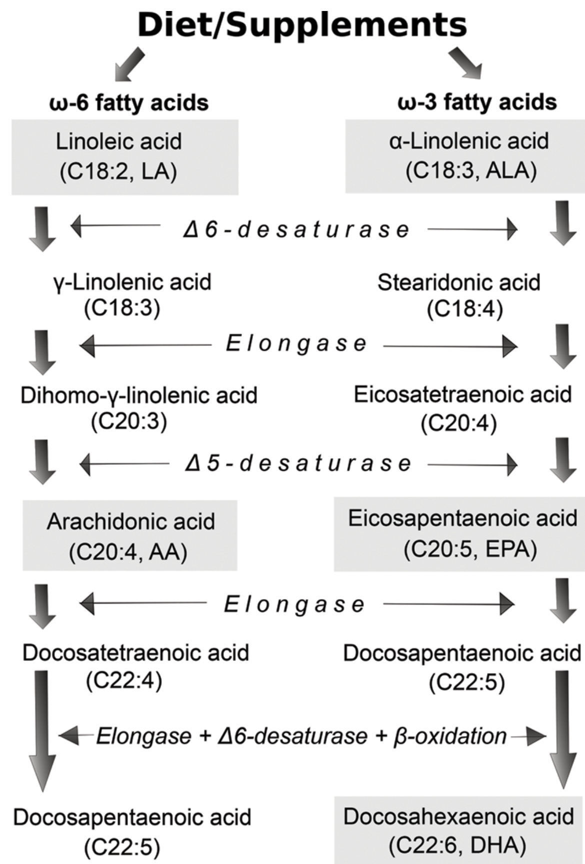

Figure 2: Biosynthesis of long-chain omega-6 and omega-3 polyunsaturated fatty acids from dietary commodities following an elongase/desaturase array in fishes. Enzym activities can be calculated based on product-to-precursor ratios of individual measurement of fatty acids [18-20].

Following earlier performed methods [18], we calculated activity of desaturases and elongases of the Cholesterylesters (ChE) of the eel tissues (muscle & gonad) -using the product-to-precursor ratios of individual LC-MS measured fatty acids- enzymatic activities as follows:

- C18:3n6/C18:2n6 ratio = Δ6-desaturase [18];
- C20:3n6/C18:3n6 ratio = elongase [18];
- C20:4n6/C20:3n6 ratio = Δ5-desaturase [18];
- C16:1*n7*/C16:0 ratio = Δ9-desaturase [18];
- C20:4/C18:2 = lineoyl CoA-desaturase [21];
- C18:0/C16:0 ratio [22];
- C18:1/C18:0 ratio [22];
- C22:5n3/C20:5n3 ratio = elongase [18];
- C22:6n3/C22:5n3 ratio = [elongase+Δ6-desaturase+β-oxidation] [18].

Calculations and statistics

Biomarker: A definition for a biomarker in this study is arbitrarily chosen following: biomarker= (> 200% in the [migrant] / [pre-migrant] ratio calculation.

For all parameter, the mean value of the pre-migrant eel group was compared to the mean value of the migrant group. Statistics were performed via SPSS [23], using a two-tailed T-Test for differences between the pre-migrant group and migrant eel group. P≤005 was considered as statistically significant. Normality of the data and homogeneity of variances were checked by Kolmogorov-Smirnov and F_{max} tests, respectively. Principal Component Analysis (PCA) was carried out on the parameters of lipid metabolism measured via reversed phase liquid chromatography coupled to mass spectrometry. This type of analysis allows one to simultaneously examine the relative state of individuals according to three or more variables. We used Principal Component Analysis (PCA) statistical methods, which are specially developed, for application in biomedical research [24,25] using TNO IMPRESS, EQUEST and WINLIN software.

Principal Components Analysis (PCA) is a classic statistical technique used to reduce multidimensional data sets to lower dimensions for analysis [26]. The applications include exploratory data analysis and data for generating predictive models. PCA involves the computation of the eigenvalue decomposition or singular value decomposition of a data set, usually after mean centering the data for each attribute.

Results

An example of an LCMS chromatogram of muscle tissue (left panel) and gonad tissue (right panel) is displayed in Figure 3. Three groups of chemical compounds can be clearly distinguished in these Figures: A) after 9-11 minutes retention time the Lysophosphatidylcholines (LPC) become visible with at 12 minutes the Internal Standard di-lauroyl-phoshatidylcholine (IS); B) after 13-16 minutes the phosphatidylcholines (PC), Sphingomyelins (SPM), Diacylglycerols (DG) and Phosphatidylethanolamines (PE) become visible; and C) after 17-19 minutes the Triacylglycerols (TG) and Cholesteryl-esters (ChE).

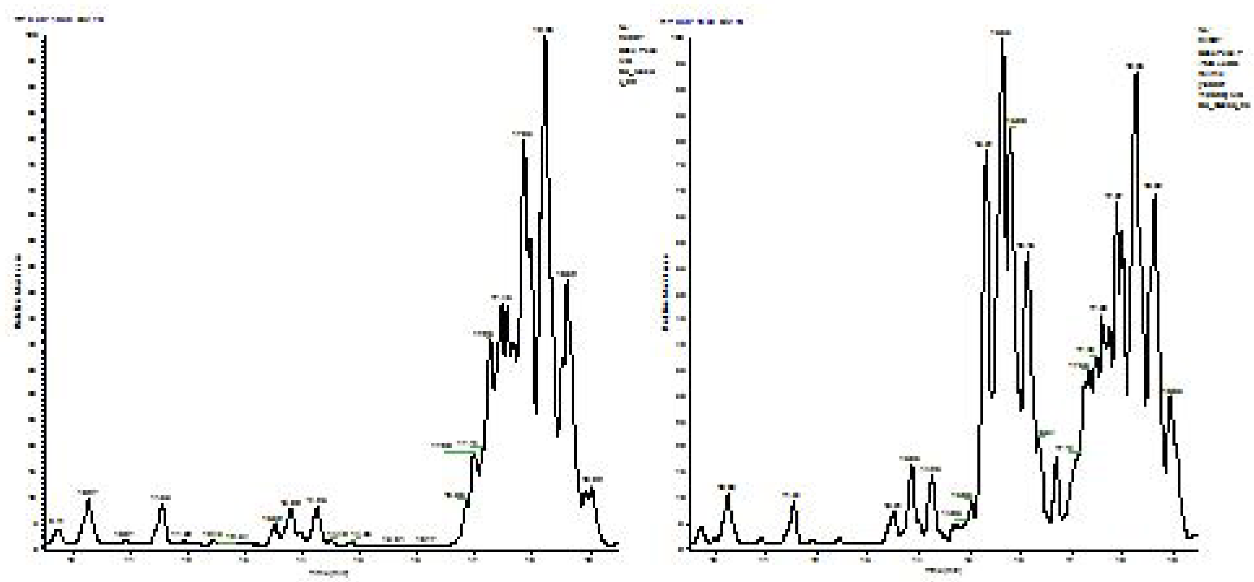

Figure 3: Lipid profiles determined by reversed phase liquid chromatography coupled to mass spectrometry (LCMS) in muscle- (left) and gonad- (right) homogenate of a migratory female eel (silver) with a length of around 68 cm and a GSI of 1.32. Principle of the method is separation based on mass and polarity.

Detailed read out and peak assignment are given in Annex 1 for muscle tissue and in Annex 2 for gonad tissue. In this study we observed clearly tissue specific differences between muscle and gonad tissue. Lipid composition and dynamics (comparison yellow vs. silver) from the lipid fraction like Phosphatidylcholine (PC), Sphingomyelin (SPM), Diacylglycerols (DG), Lysophosphatodylcholine (LPC), Cholesterylesters (ChE), Triacylglycerols (TG) were determined by LCMS measurements of around 150 lipid compounds per tissue. Phospholipids (LPC, SPM, DG, PC, PE, ChE) and Triacylglycerols (TGs) (see Annex 1 & 2). From these figures but also from the mean±STD of the individual molecular data given in Table 1 and Annex 1 & 2 it becomes clear that muscle tissue is extremely rich in TGs while in gonad tissue both TGs and ChE are the most abundant lipids.

Table 1: LC-MS spectra for molecular lipid measurements in the muscle and gonad tissue of a “yellow” (pre-migrant) and a “silver” (migrant) eel group.

Muscle	Migrant (n=7)	Pre migrant (n=6)	T-Test
LPC	0.032±0.0137 (0.0480%)	0.033±00010 (0.0497%)	P≤0.876
SPM	0.0541±0.014 (0.0812%)	0.053±0.0156 (0.0799%)	P≤0.859
DG	0.215±0.0905 (0.323%)	0.176±0.0644 (0.265%)	P≤0.380
PC	3.503±1.1662 (5.26%)	3.294±0.9139 (4.96%)	P≤0724
PE	0.079±0.0167 (0119%)	0.086±0.0261 (0.265%)	P≤607
ChE	2.962±1.025 (4.45%)	3.103±1.487 (4.68%)	P≤0.855
TG	59.756±16.932 (89.72%)	59.612±17.331 (89.84%)	P≤0.988
SUM LIPIDS	66.601 (100%)	66.357 (100%)	
EPA (C20-5-ChE)	0.010±0.0047 (0.015%)	0.007±0.0055 (0.011%)	P≤0.374
DHA (C22-6-ChE)	0.024±0.0090 (0.036%)	0.020±0.01323 (0.030%)	P≤0.503
AA (C20-4-ChE)	0.004±0.00299 (0.006%)	0.002±0.00153 (0.003%)	P≤0.272

Gonad (ovary)	Migrant (n=7)	Pre migrant (n=6)	T-Test
LPC	0.269±0.0407 (0.243%)	0.215±0.176 (0.221%’)	P≤0.490
SPM	0.060±0.0076 (0.0545%)	0.064±0.0191 (0.066%)	P≤0.636
DG	1.183±0.3421 (1.066%)	0.903±0.3708 (0.929%)	P≤0.188
PC	7.416±1.008 (7.438%)	3.571±1.730 (3.676%)	P≤0.0015 ***
PE	0.255±0.0799 (0.230%)	0.204±0.0490 (0.210%)	P≤0.194
ChE	2.281±0.278 (2.056%)	2.743±0.525 (2.823%)	P≤0.092
TG	99.704±18.609 (89.88%)	89.452±48.003 (92.07%)	P≤0.639
SUM LIPIDS	110.926 (100%)	97.152 (100%)	
EPA (C20-5-ChE)	0.0139±0.0525 (0.013%)	0.0124±0.1029 (0.013%)	P≤0.756
DHA (C22-6-ChE)	0.056±0.0238 (0.0234%)	0.056±0.0408 (0.0576%)	P≤0.991
AA (C20-4-ChE)	0.032±0.0150 (0.0288%)	0.0034±0.0360 (0.004)	P≤0.895

From Table 1 we can see all seven major lipid classes occur in both muscle and gonadal tissue. TGs for almost 90% in muscle tissue followed by PC around 5%, ChE around 4.5% and other fractions below 1%. Gonadal tissue (ovary) shows a very different pattern. TGs are indeed in migrants also around 90%, while in pre-migrants they are higher around 92% with a huge variation, which makes this non-significant. PCs in ovary tissue are the next most important class of around 7.5% in pre-migrants and with a noticeable significant decrease (P≤0.0015) to around 3.7% in the migrant group. The ChE are around 2.1% among the migrants and 2.8% among the pre- migrants. The other lipid fractions are around 1% or lower. In summary, the muscle tissue is the most uniform in its composition with few differences between (pre) migrants and migrants, whereas for ovary tissue this shows more variation between the two groups. The most noticeable features are the strong and significant decrease of the PC and the stronger decrease of TGs in the pre-migrants with a huge variation between animals. In addition, the values for EPA, DHA and Arachidonic Acid (AA) are also given. EPA and DHA occur to a small extent in both types of tissue, with EPA in the range 0.01-0.02% and DHA 0.02-0.06%.

In order to stipulate the "uniqueness" of each animal based on lipid biochemistry at first, a PCA was performed on the around 100 molecular lipid compounds in muscle tissue of the 5 earlier mentioned major lipid classes. The results of a PCA are usually discussed in terms of scores and loadings. The score and loading vectors give a concise and simplified description of the variance present in the dataset. A principal component is a linear combination of the original variables (in this case: lipid concentrations) and the magnitude of its eigenvalue is a measure of the explained variance. Typical only a few principal components are required to explain >90% of the total variance in the data. In other words, PCA is a dimension reduction method, e.g. from >100 lipid attributes in the data to only four principal components, which simplifies data visualization. A clear PCA calculation was performed in this way, separating the individual animals, and depicted in Figure 4.

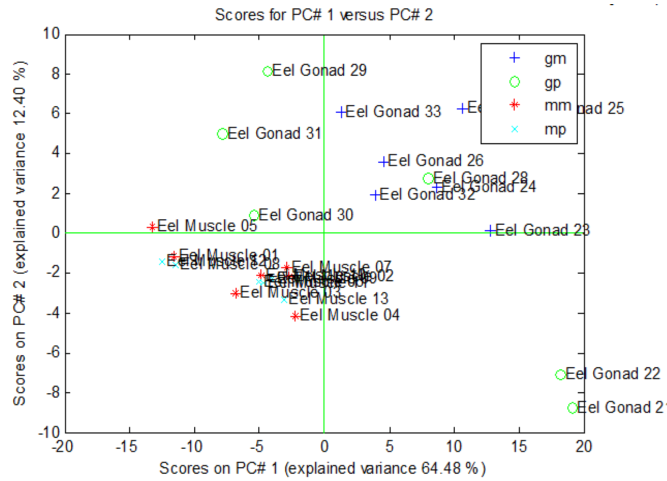

Figure 4: PCA muscle and gonad eel migrant (m) and pre-migrant (p) of the European eel (*Anguilla anguilla* L); abbreviations: gm: gonad-migrant; gp: gonad-premigrant; mm: muscle-migrant; mp: muscle-premigrant; all animals were in the range > 200 g and defined as females.

Biomarkers

It becomes clear that muscle tissue is extremely rich in TGs while in gonad tissue both TGs and ChE are the most abundant lipids followed by PC. Che are interesting because of the ability to calculate based on precursor-product ratio´s enzymatic activity (Table 2). For this reasoning we will search for biomarkers solely in the TGs and PC fraction. Remind that because of the followed Material & Methods procedure the outcomes of the different samples are dimensionless but mutually comparable. As described under Material & Methods we defined specific biomarkers for the different lipid fractions were using a 200% ration for the fraction [migrant]/[pre-migrant]*100%: Ovary PCs: C30-1 208.9%; C32-2 331.81; C34-2 275.0% ; C34-3 338.2%; C34-5 230.0%; C36-2 241.8%; C36-4 215.0%; C36-5 211.2%; C36.6% 280.3%; C38-6 203.4%; C38-7 272.2%; C40-7 214.3%; Muscle TGs: none; Ovary PCs: C34-0 215.9%; ChE C18-3 223.7%; Ovary TGs: C46-4 232.37%; C46-5 308.0%; C48-6 266.3%; C52-9 258.2% (see also Annex 1 & 2).

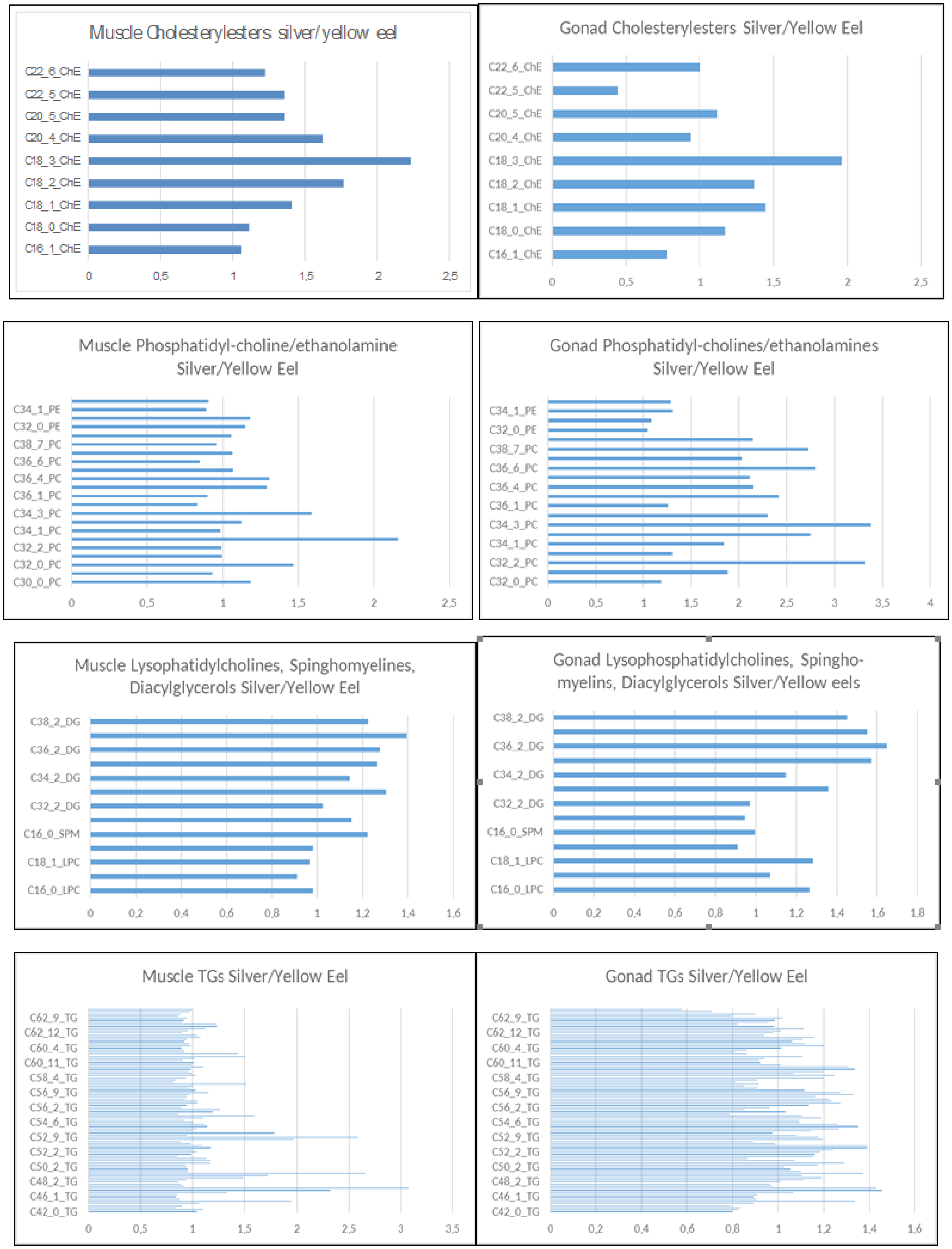

Figure 5: Search for biomarkers for muscle and gonad (ovary) tissue for European eel eel for the 6 major lipid compounds Phosphatidylcholine (PC), Sphingomyelin (SPM), Diacylglycerols (DG), Lysophosphatodylcholine (LPC), Cholesterylesters (ChE), Triacylglycerols (TG).

Table 2: Comparison between a (pre)migrant and a migrant group of the European eel for the Cholesterylester lipid fractions in muscle and gonad tissue and its from the elongase/desaturase derived enzymatic activities based on product/precursor ratios.

LCMS Compound			
MUSCLE	**migrant (n=7)**	**(pre)migrant (n=6)**	**P-value**
C16-1 Che	0.003±0.0007	0.003±0.0005	P≤0.667
C18-0 Che	0.024±0.0046	0.022±0.0041	P≤0.319
C18-1 Che	0.029±0.0073	0.020±0.0038	P≤0.0257*
C18-2 Che	0.002±0.0007	0.001±0.0003	P≤0.0380*
C18-3 Che	0.002±0.0015	0.001±0.0002	P≤0.1784
C20-4 Che	0.004±0.0030	0.002±0.0015	P≤0.2722
C20-5 Che (EPA)	0.010±0.0047	0.007±0.0055	P≤0.3743
C22-5 Che	0.012±0.0056	0.0009±0.0051	P≤0.3248
C22-6 Che (DHA)	0.024±0.0090	0.020±0.0132	P≤0.5030
Enzymatic activity			
C18-0/C18-1	0.880±0.2046	1.092±0.2308	P≤0.1123
C18-3/C18-2	0.799±0.3778	0.732±0.1703	P≤0.6816
C20-3/C18-3	n.d.	n.d.	-
C20-4/C20-3	n.d.	n.d.	-
C16-1/C16-0	n.d.	n.d.	-
C20-4/C18-2	2.059±0.8511	2.287±1.3359	P≤0.7285
C22-5/C20-5	1.187±0.22927	1.507±0.9196	P≤0.4449
C22-6/C22-5	2.308±0.9523	2.307±0.7068	P≤0.9981
GONAD	**migrant (n=7)**	**(pre)migrant (n=6)**	
C16-1 Che	0.020±0.0103	0.025±0.0263	P≤0.6416
C18-0 Che	0.056±0.0228	0.048±0.0233	P≤0.5394
C18-1 Che	0.296±0.1038	0.205±0.1569	P≤0.2557
C18-2 Che	0.030±0.00190	0.022±0.0229	P≤0.5078
C18-3 Che	0.021±0.0112	0.011±0.0100	P≤0.1021
C20-4 Che	0.032±0.0150	0.034±0.0360	P≤0.8951
C20-5 Che (EPA)	0.139±0.0525	0.124±0.1029	P≤0.7567
C22-5 ChE	0.043±0.0227	0.097±0.1209	P≤0.3257
C22-6 ChE (DHA)	0.056±0.0238	0.056±0.0408	P≤0.9906

Enzymatic activity			
C18-0/C18-1	0.188±0.0136	0.395±0.3501	P≤0.2071
C18-3/C18-2	0.769±0.3638	0.577±0.1339	P≤0.2308
C20-3/C18-3	n.d.	n.d.	-
C20-4/C20-3	n.d.	n.d.	-
C16-1/C16-0	n.d.	n.d.	-
C20-4/C18-2	0.954±0.20332	1.543±0.2506	P≤0.00132**
C22-5/C20-5	0.303±0.0800	0.774±0.5678	P≤0.0979
C22-6/C22-5	1.387±0.3201	1.381±0.833`	P≤0.9865

Based on product-precursor ratios we calculated following a Systems Biology approach muscle- and gonad- enzymatic activities. Desaturase (C16:1/C16:0), Δ6-desaturase (C18:3/C18:2), elongase (C22:5/C20:5), [elongase+Δ6-desaturase+β-oxidation] (C22:6/C22:5) and lineoyl CoA-desaturase (C20:4/C18:2) could be calculated. For lineoyl CoA-desaturase for the gonad between pre-migrants 1.543±0.2506 and migrants 0.954±0.2033 the enzymatic activity decreased significantly (P≤0.00132**) which is indicative for a 1.7 fold decrease of this enzyme in the gonad of the silver eel. Lineoyl CoA-desaturase plays a key role in the turnover of 18:2(n-6) to 20:4(n-6) and consequently determines the total amount of PUFAs.

In Figure 6 T2-weighted images are given showing the TGs are closely located long the muscle tissue in the adults.

Figure 6: T2-weighted MRI images of a European eel (*Anguilla anguilla*) of ≈120 gram. The T2-weighted areas are White Adipose Tissue (WAT) consisting mainly out of Triacylglycerols (TGs). (Courtesy: Prof.dr. Klaas Nicolay, Technical University, Eindhoven). Due to the large fat depots directly close to the myotomes eels are able to swim such tremendous distances to their spawning grounds without feeding.

Discussion

In this study the lipid and fatty acid composition of the muscle and gonads of female European freshwater eel *Anguilla anguilla* from was determined in migratory silver- (n=7) and pre-migratory (yellow) stages (n=6) for muscle composition and gonad composition by LCMS measurements. From morphological observations at body coloring, size and position of the eyes, and size of the pectoral fin a clear distinction could be made between silvering and non-silvering stages (Figure 1). Lipid composition and dynamics (comparison yellow *vs.* silver) from the lipid fraction like Phosphatidylcholine (PC), Sphingomyelin (SPM), Lysophosphatodylcholine (LPC), Cholesterylesters (ChE), Triacylglycerols (TG) were determined by LCMS measurements of around 150 lipid compounds per tissue. Phospholipids (LPC, SPM, DG, PC, PE, ChE) and Triacylglycerols (TGs) (see Annex 1 & 2). In addition, the values for EPA, DHA and Arachidonic Acid (AA) are also given. At first, a PCA was performed on the around 140 molecular lipid compounds in gonad tissue and also around 140 molecular compounds in muscle tissue of the 5 earlier mentioned major lipid classes. The results of a PCA are usually discussed in terms of scores and loadings. The score and loading vectors give a concise and simplified description of the variance present in the dataset (26). A principal component is a linear combination of the original variables (in this case: lipid concentrations) and the magnitude of its eigenvalue is a measure of the explained variance. Typical only a few principal components are required to explain >90% of the total variance in the data. In other saying PCA is a dimension reduction method, e.g. from >100 lipid attributes in the data to only a four principal components which simplifies data visualization. From our observations at the two groups of (pre) migrant and migrant for gonad- and muscle- tissue the scores on PC#1 (horizontal axis) could explain 64.48% of the variance while the scores on PC#2 (vertical axis) could explain 12.4% of the variance.

Despite the fact that we know a large proportion of the factors involved in the silvering process, it is still a black box. Silvering changes are involved in the physiological changes in terms of osmoregulation and high-pressure resistance. It has no effect on swimming performance but has a strong effect on maturation or males and females. We assume -as indicated with 5,500 km swim experiments with females [27] in the Blazka swim-tunnels [28] swimming has a positive effect on silvering, probably because it influences maturation [29]. However, the factors involved in lipogenesis and lipolysis remain completely unclear for the European eel despite an extensive study on the Japanese eel [16] and the shortfinned Australian eel [17].

At the cellular/organ/tissue level of the organism one of the challenges of measuring metabolite levels within the research area "Metabolomics approach in animal metabolism" is determining the significance of it is objectively measured and evaluated as an indicator of normal biological processes, nutritional intervention, pathogenic processes, or pharmacological responses to a therapeutic intervention: exercise, nutritional and metabolomic changes. Metabolomics is a discipline dedicated to the systematic study of small molecules (i.e. metabolites) in cells, tissues, and different bio-fluids. Metabolite levels can be regarded as amplified responses of biological systems to genetic or environmental changes [30]. The challenge of a metabolomics approach is not only the discovery of changes in metabolite profile but it is figuring out what these changes mean which ultimately can lead to a biomarker for a pathogenesis or an ecological or environmental change. By a systems-biology approach [31] and the search for new ecological biomarkers applicable for "silvering of eel" this can lead to an "Individual Animal Approach". This would provide a scientific baseline for the research area of artificial reproduction area for individual treatment of female spawners with hormones (see Perspectives).

A particular area of interest of a lipidomics based Systems Biology, will be identification of novel ecological biomarkers that can be used in the assessment of the metamorphose stage of the eels during the silvering process. A biomarker is defined as a substance used as an indicator of a biological state. The search for reliable applicable biomarkers for silvering in eel studies provide a scientific baseline in "Individual Animal Approach" for defining silvering in eels but more important for individual female spawners (see Perspectives). In this respect a demand for a biomarker of the lipid fraction is that homeostatic conditions are needed for the biomarker to reflect accurately long-term intake and not to be biased by lipolysis [32]. Fatty acids can be measured as free fatty acids in serum, components of circulating triacylglycerols, components of erythrocyte membranes, phospholipids or cholesteryl esters, or adipose tissue from various sites. For the fatty eel the gonad and muscle tissue are therefore of particular interest. So we measured around 140 TGs in muscle and 141 TGs in gonad tissue (Annex 1 & 2) of which eighteen were indicated as biomarker (> 200% in the [migrant]/[pre migrant] ratio calculation.

TGs are highly concentrated stores of metabolic energy because they are reduced and anhydrous. When energy is required during the stressful 5,500 km spawning migration of the European eel the hormones adrenaline and glucagon stimulate triacylglycerol mobilization by activation of hormone-sensitive lipase in adipose tissue, and fatty acids and glycerol are released. The fatty acids are bound to albumin and transported in the blood to the tissues for oxidation e.g. by muscle. The glycerol is converted by the liver to glucose, which in turn is released for oxidation, especially by the red blood cells and brain, neither of which can use fatty acids as a respiratory fuel [33,34]. There is a continuous cycling and redistribution of non-oxidized fatty acids between different organs, especially in the post-absorptive state, with a central role for the liver and the adipose tissue [35]. Just like in mammals we hypothesize in absorptive state (pre-migratory stage) triacylglycerols from the White Adipose Tissue (WAT) stores are transported by the blood to the peripheral organs and mainly muscles in the form of chylomicrons. An enzyme that is produced in peripheral organs called lipoprotein lipase (LPL) is required for the intravascular hydrolyse of chylomicrons into fatty acids. These can be taken out of the blood. LPL is stimulated by insulin, especially in adipose tissue, and by exercise, especially in muscle tissue. Remnant particles that are not hydrolysed are transported to the liver.

In the post-absorptive (fasting) state -migratory stage- the whole triacylglycerol metabolism is the other way round. Triacylglycerols that are contained in WAT tissue (Figure 7), are continuously hydrolysed by an enzyme called Hormone Sensitive Lipase (HSL). HSL in the fed state is inhibited by insulin. Most of the generated free fatty acids are released into the blood and transported to other organs where they can be used as energy substrate. FFA release generally exceeds demand, especially in resting conditions. The liver takes up a considerable amount of these FFAs, which are then oxidized or re-esterified into triacylglycerols (Figure 7). In addition, the results of the study of [17] at the triacyglycerol physiology in the short-finned *Anguilla australis* throughout early oogenesis suggested that increased hepatic apolipoprotein B production is a conserved vertebrate response to prolonged period of fasting.

Many cell types and organs have the ability to synthesize triacylglycerols, but in animals the liver and intestines are most active, although most of the body stores of the lipid are in adipose tissue. Only the Phosphatidylcholine content of the gonad of the migratory animals was for the total amount significantly higher (P≤0.0015) in comparison to pre-migratory stages.

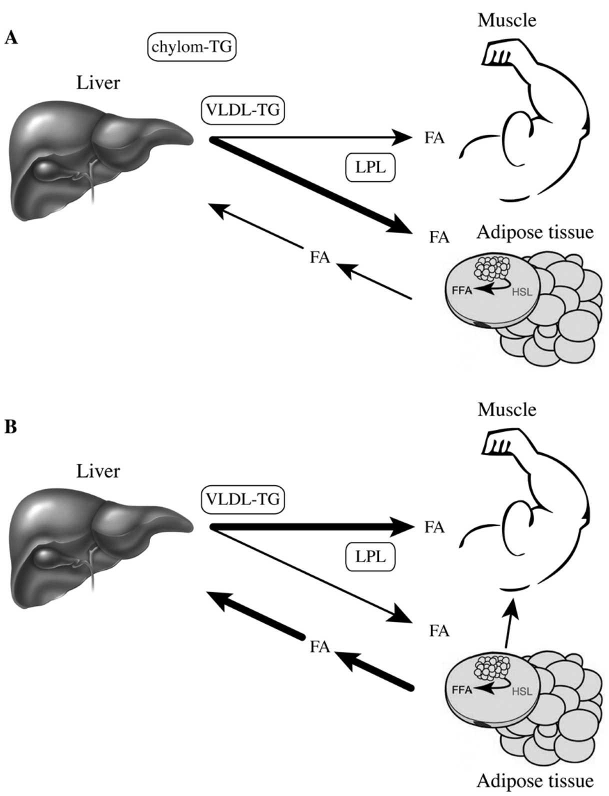

Figure 7: Diversion of fatty acid to peripheral tissues in mammals. **A:** In the fed state, chylomicrons are lipolysed by Lipoprotein Lipase (LPL) to generate fatty acids (FA) that are mainly taken-up by muscle and adipose tissue for oxidation and esterification into TGs, especially in the adipose tissue. **B:** In the fasting state, TGs within the adipose tissue are lipolysed by the enzyme Hormone-Sensitive Lipase (HSL) and fatty acids are released into the blood in excess of oxidative requirements. The excessive fatty acids can be taken-up by the liver.

Note: The arrows indicate the fluxes of fatty acids. FA indicates fatty acids; LPL, lipoprotein lipase; HSL, hormone-sensitive lipase; VLDL, very-low-density lipoprotein; chylom, chylomicrons derived from the intestine.

Fatty acid desaturase appears in all organisms: for example, including humans. More specific four desaturases occur in humans: Δ^9 desaturase, Δ^6 desaturase, Δ^5 desaturase, and Δ^4 desaturase [36]. Δ^9 desaturase, also known as stearoyl-CoA desaturase-1, is used to synthesize oleic acid, a monounsaturated, ubiquitous component of all cells in the human body. Δ^9 desaturase produces oleic acid by desaturating stearic acid, a saturated fatty acid either synthesized in the body from palmitic acid or ingested directly. The lipid composition of cellular membranes is regulated to maintain membrane fluidity. A key enzyme involved in this process is the membrane-bound stearoyl-CoA Desaturase (SCD) which is the rate-limiting enzyme in the cellular synthesis of monounsaturated fatty acids from saturated fatty acids. A proper ratio of saturated to monounsaturated fatty acids contributes to membrane fluidity. Alterations in this ratio have been implicated for humans in various disease states including cardiovascular disease, obesity, non-insulin-dependent diabetes mellitus, hypertension, neurological diseases, immune disorders, and cancer [37].

The polar lipids 18:2n-6 and 20:4n-6 are characteristically contained at high levels in freshwater fishes and are therefore useful biomarkers for freshwater- or herbivorous fish species such as common carp [38], goldfish [38], rainbow trout [39], eel [39] and Indian featherback fish [16,40]. In contrast low levels of n-6 PUFAs are generally found in marine fish species except for some herbivorous fish species that prefer and consume seaweed and specifically accumulate 2-:4n-6 in their tissue lipids [16]. In previous studies we demonstrated that seaweeds in general are rich in PUFAs especially the n-9 and n-3 fraction but not the n-6 [41], what consequently can explain the characteristic low levels of n-6 PUFAs in marine fish in great extent [16].

What is so characteristic of this study is that the European eel (*Anguilla anguilla*) - despite the fact that it has a marine phase as freshwater phase - contains high levels of the specific biomarker C18: 2n-6 which is so characteristic for freshwater fish [38-40]. The extreme high concentrations for this compound are similar to the observations of [16] for the Japanese eel (*Anguilla japonica*). C18:2n-6 is thus an important ecological biomarker for European eel which shows characteristic lipid profiles of freshwater fishes even prior to catadromic migration in the ocean towards its assumed spawning grounds 5,500 km away in the Sargasso Sea [1,2]. This can from ecological perception solely be explained because they have an extreme longlife phase 7-20 years in the freshwater, lakes and rivers hunting prey fish and other freshwater species [42].

The limited long-chain n-3 PUFAs EPA & DHA are mainly found in marine fish in higher trophic levels. In particular, DHA is the only major PUFA in highly migratory fishes [43,44]. This means that both EPA & DHA are terminal PUFAs and many marine fishes only accumulate them [43,45]. Interestingly, unusually high levels of 22:5n-3 were found in polar lipids of all *A. japonica*. This is the specific lipid profile for *A. japonica* because only some mollusks, such as abalone and snails [46,47] show noticeable levels of 22:5n-3 (docosapentaenoic acid). High levels of 22:5n-3 in *A. japonica* lipids indicate its biosynthetic weakness of DHA similar to those in the mollusks [47,48] and other freshwater fishes [39,48]. Our observations at *A. anguilla* indicate docosapentaenoic acid (22:5n-3 Che) is for migrants (not significantly) higher in muscle tissue with 135.6 % while it is for ovary tissue (not significantly) lower with 44.3%.

In addition, we have undertaken to study the PUFA synthesis pathway in fish for two reasons. First, fish are an important source of PUFA, especially of the long chain C20 and C22n-3 PUFAs which are important for a proper brain development in humans [49] which either are deficient in human diets [50] either show an imbalanced Ω6 / Ω3 ration [41]. Second there is a wide variation between fish species to synthesize PUFA [51, 52]. Many freshwater species such as trout, tilapia, and carp are able to convert dietary C18 precursor fatty acids to arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid. However, marine species such as turbot and sea bream, which are inherently piscivorous, have very limited abilities to perform these conversions [51, 52]. European eel, *A. anguilla* shows the characteristics of freshwater fish and are able to convert dietary C18 precursor fatty acids to arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid.

Perspectives: Because the eel is an endangered species in the very serious category "Critically Endangered" (CR) [14] (see introduction) artificial hormone techniques [53,54] are interesting tools to safeguard a healthy population. However, the percentages of obtaining successful larvae from an initial artificial hormone treated female population are low and are varying between around 2-5% (unpublished results). Therefore, specific blood maturation lipidomics related biomarkers for female are in this way a tool for an "Individual spawner treatment". By performing such approach in combination with refined and improved hormone protocols [54] this fish species can be protected.

Annex 1: Measured compounds in gonad (ovary) of eel with LC-MS techniques like Lysophosphatidylcholines (LPC), Phosphatidylcholine (PC), Spingomyelin (SPM), Diacylglycerols & Triacylglycerols (TG).

Compound	Migrant n=7	Pre-migrant n=6	T-test (P-value)	Change%
C16-0-LPC	0.146±0.0244	0.1154±0.1093	0.529	126.53
C18-0-LPC	0.0225±0.0032	0.021±0.0147	0.8209	106.91
C18-1-LPC	0.1012±0.0183	0.0789±0.0546	0.3757	128.29
LPC	0.2697±0.0407	0.2153±0.1762	0.4903	125.26
C14-0-SPM	0.0401±0.0049	0.0441±0.0158	0.5699	90.84
C16-0-SPM	0.0202±0.0035	0.0203±0.0052	0.9666	99.47
SPM	0.0603±0.0076	0.0644±0.0191	0.6354	93.57
C32-1-DG	0.1416±0.0338	0.1499±0.0759	0.8127	94.49
C32-2-DG	0.0437±0.0108	0.045±0.0149	0.8634	97.12
C34-1-DG	0.3423±0.1172	0.2521±0.1438	0.2493	135.75
C34-2-DG	0.1877±0.0438	0.1631±0.0538	0.3927	115.08
C36-1-DG	0.101±0.0329	0.0644±0.0313	0.0654	156.7
C36-2-DG	0.2654±0.0901	0.1613±0.061	0.0321	164.61
C36-3-DG	0.0679±0.0206	0.0438±0.0103	0.0236	154.91
C38-2-DG	0.0338±0.0108	0.0233±0.0088	0.0779	145.3
DG	1.1835±0.3421	0.903±0.3708	0.1881	131.06
C30-0-PC	0.035±0.0059	0.0349±0.0044	0.9773	100.24
C30-1-PC	0.0281±0.0106	0.0134±0.007	0.0135	208.89

C32-0-PC	0.0613±0.0115	0.0518±0.0105	0.1482	118.35
C32-1-PC	0.4073±0.078	0.2162±0.071	0.0008	188.36
C32-2-PC	0.0987±0.0383	0.0297±0.02	0.0023	331.81
C34-0-PC	0.0153±0.0071	0.0117±0.0065	0.37	130.22
C34-1-PC	1.5071±0.1894	0.8169±0.2979	0.0011	184.48
C34-2-PC	0.4774±0.0922	0.1736±0.0902	0.0001	274.99
C34-3-PC	0.1153±0.0426	0.0341±0.0192	0.0016	338.21
C34-5-PC	0.0394±0.0117	0.0171±0.0125	0.0077	229.95
C36-1-PC	0.1118±0.0232	0.0889±0.0199	0.0819	125.76
C36-2-PC	0.3061±0.0778	0.1266±0.0666	0.0009	241.77
C36-4-PC	0.7923±0.4668	0.3685±0.1566	0.0559	215
C36-5-PC	1.1729±0.1566	0.5554±0.333	0.0044	211.17
C36-6-PC	0.2271±0.0584	0.081±0.0675	0.002	280.31
C38-6-PC	1.5116±0.4252	0.7431±0.4641	0.011	203.43
C38-7-PC	0.2557±0.0777	0.094±0.0821	0.0043	272.16
C40-7-PC	0.2435±0.0588	0.1136±0.0775	0.008	214.29
PC	7.406±1.0083	3.5709±1.7296	0.0015	207.4
C32-0-PE	0.0248±0.0071	0.0238±0.0048	0.7573	104.42
C34-0-PE	0.0216±0.013	0.02±0.0071	0.7869	107.96
C34-1-PE	0.1088±0.0302	0.0835±0.0204	0.1021	130.28
C36-1-PE	0.0996±0.0343	0.0771±0.0238	0.1947	129.08
PE	0.2547±0.0799	0.2044±0.049	0.1945	124.63
C16-1-ChE	0.0196±0.0103	0.0252±0.0263	0.6416	77.87
C18-0-ChE	0.0562±0.0228	0.048±0.0233	0.5394	116.96
C18-1-ChE	0.2964±0.1038	0.2049±0.1569	0.2557	144.69
C18-2-ChE	0.0303±0.019	0.0222±0.0229	0.5078	136.46
C18-3-ChE	0.0214±0.0112	0.0109±0.01	0.1021	196.2
C20-4-ChE	0.0318±0.015	0.034±0.036	0.8951	93.65
C20-5-ChE	0.1392±0.0525	0.1242±0.1029	0.7567	112.04
C22-5-ChE	0.0431±0.0227	0.0974±0.1209	0.3257	44.27
C22-6-ChE	0.0561±0.0238	0.0558±0.0408	0.9906	100.41
ChE	0.6941±0.2594	0.6226±0.5257	0.7706	111.48
C42-0-TG	0.0414±0.0174	0.0519±0.0464	0.6181	79.75
C42-1-TG	0.0199±0.0099	0.0242±0.0232	0.6875	82.32
C44-0-TG	0.184±0.0326	0.2204±0.1422	0.564	83.48
C44-1-TG	0.2926±0.0954	0.3686±0.3122	0.5881	79.4
C44-2-TG	0.0777±0.0297	0.0872±0.0826	0.7965	89.03
C44-3-TG	0.0174±0.0108	0.013±0.0121	0.512	133.33
C46-0-TG	0.3425±0.052	0.3802±0.1898	0.6552	90.09
C46-1-TG	1.0994±0.1962	1.2321±0.7215	0.6785	89.23

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C46-2-TG	0.9077±0.2514	1.0014±0.7583	0.782	90.64
C46-3-TG	0.2435±0.1127	0.2283±0.2086	0.8773	106.67
C46-4-TG	0.0786±0.0484	0.054±0.0517	0.3983	145.58
C46-5-TG	0.0149±0.0132	0.0105±0.0115	0.5267	142.87
C48-0-TG	0.4629±0.0606	0.4747±0.1717	0.8781	97.52
C48-1-TG	2.0174±0.258	2.0926±0.9891	0.8628	96.41
C48-2-TG	2.6301±0.4129	2.6042±1.4385	0.9674	101
C48-3-TG	1.5376±0.3757	1.3844±0.929	0.7175	111.06
C48-4-TG	0.6947±0.3122	0.5824±0.5003	0.6465	119.29
C48-5-TG	0.2817±0.1514	0.2555±0.2532	0.83	110.26
C48-6-TG	0.0504±0.0325	0.0367±0.0379	0.5051	137.2
C50-0-TG	0.6021±0.1243	0.5474±0.3324	0.7162	109.98
C50-1-TG	2.9817±0.4175	2.8268±1.1347	0.762	105.48
C50-2-TG	4.1364±0.9502	4.0213±1.807	0.8922	102.86
C50-3-TG	3.0879±0.5141	2.6324±1.4158	0.4827	117.31
C50-4-TG	1.924±0.6837	1.4937±0.8999	0.3623	128.81
C50-5-TG	1.6428±0.5226	1.5285±1.1778	0.8325	107.48
C50-6-TG	0.708±0.3038	0.8185±0.7777	0.7542	86.51
C52-0-TG	0.6625±0.1975	0.5752±0.4729	0.6871	115.16
C52-1-TG	3.4504±0.7457	2.9784±1.9116	0.5892	115.85
C52-2-TG	5.8141±0.9056	4.9162±2.1127	0.3675	118.26
C52-3-TG	3.7321±0.5668	3.0095±1.4442	0.2911	124.01
C52-4-TG	2.2053±0.8243	1.5878±0.7369	0.1817	138.9
C52-5-TG	2.7526±0.9433	1.9802±1.1347	0.2164	139.01
C52-6-TG	2.784±0.5388	2.8223±1.8362	0.9623	98.64
C52-7-TG	1.2382±0.4713	1.3918±1.2325	0.7828	88.96
C52-8-TG	0.2955±0.1542	0.2474±0.2464	0.69	119.42
C52-9-TG	0.075±0.0454	0.0638±0.0647	0.7304	117.57
C54-1-TG	1.846±0.5711	1.7056±1.4606	0.8317	108.24
C54-10-TG	0.1656±0.0806	0.1698±0.1689	0.9571	97.52
C54-2-TG	4.5117±0.91	3.9481±2.5821	0.6292	114.28
C54-3-TG	3.6694±0.7335	2.9009±1.5046	0.2916	126.49
C54-4-TG	1.4373±0.4838	1.0654±0.3798	0.1493	134.91
C54-5-TG	2.1451±0.8722	1.703±0.629	0.3133	125.96
C54-6-TG	3.4629±0.6107	3.1716±1.4274	0.6571	109.19
C54-7-TG	3.0549±0.4363	2.801±1.5072	0.7047	109.06
C54-8-TG	1.5709±0.332	1.3202±0.9008	0.5421	118.98
C54-9-TG	0.5595±0.235	0.5077±0.4577	0.8091	110.21
C56-1-TG	0.2099±0.0694	0.2672±0.2762	0.6397	78.55
C56-10-TG	0.8798±0.2506	0.851±0.6096	0.9172	103.38

C56-11-TG	0.2966±0.1339	0.3477±0.3223	0.7285	85.3
C56-2-TG	1.463±0.3819	1.5188±1.3338	0.9245	96.32
C56-3-TG	1.9318±0.4321	1.7017±1.1445	0.6577	113.53
C56-4-TG	0.7289±0.2922	0.5715±0.2491	0.3169	127.54
C56-5-TG	1.0064±0.3301	0.8174±0.2114	0.2406	123.12
C56-6-TG	2.0441±0.3047	1.6727±0.5885	0.2046	122.2
C56-7-TG	2.718±0.2399	2.3352±0.8972	0.3521	116.39
C56-8-TG	1.8778±0.2861	1.4095±0.6488	0.1479	133.22
C56-9-TG	1.1424±0.3251	0.8955±0.4745	0.3109	127.57
C58-10-TG	1.0191±0.2243	0.9142±0.3375	0.5338	111.47
C58-11-TG	0.9939±0.172	1.0913±0.5349	0.6842	91.07
C58-12-TG	0.3799±0.1316	0.4466±0.3204	0.6494	85.07
C58-13-TG	0.057±0.0308	0.0622±0.0564	0.8455	91.64
C58-2-TG	0.2459±0.0676	0.3017±0.3096	0.6817	81.49
C58-3-TG	0.5992±0.1711	0.6608±0.6189	0.8218	90.69
C58-4-TG	0.599±0.2152	0.499±0.3388	0.55	120.04
C58-5-TG	0.5932±0.2251	0.4758±0.212	0.3542	124.69
C58-6-TG	0.7894±0.1637	0.7399±0.3132	0.7373	106.69
C58-7-TG	1.3031±0.1165	1.0885±0.3604	0.2128	119.71
C58-8-TG	1.0899±0.1584	0.8163±0.2966	0.0804	133.51
C58-9-TG	0.7451±0.245	0.5697±0.1576	0.15	130.78
C60-10-TG	0.4274±0.1123	0.4234±0.1051	0.9484	100.94
C60-11-TG	0.7366±0.1016	0.7975±0.2601	0.6084	92.37
C60-12-TG	0.7117±0.1273	0.7758±0.3296	0.6686	91.73
C60-13-TG	0.2774±0.0649	0.2953±0.1682	0.8134	93.92
C60-14-TG	0.0847±0.0428	0.0765±0.0599	0.7885	110.59
C60-15-TG	0.0216±0.013	0.0251±0.0243	0.761	86.08
C60-2-TG	0.0285±0.0075	0.0351±0.0339	0.6563	81.04
C60-3-TG	0.1043±0.0291	0.1208±0.1252	0.7636	86.32
C60-4-TG	0.1439±0.0618	0.1421±0.1365	0.9767	101.29
C60-5-TG	0.2296±0.1104	0.1915±0.1377	0.5988	119.89
C60-6-TG	0.3448±0.0921	0.3094±0.212	0.7165	111.43
C60-7-TG	0.4338±0.0578	0.4089±0.2079	0.7862	106.1
C60-8-TG	0.2976±0.0481	0.2692±0.0852	0.49	110.58
C60-9-TG	0.1738±0.0729	0.1503±0.0306	0.4586	115.64
C62-10-TG	0.1028±0.0322	0.1097±0.0267	0.6819	93.72
C62-12-TG	0.3205±0.059	0.328±0.1142	0.8889	97.72
C62-13-TG	0.2251±0.0557	0.2223±0.096	0.9523	101.24
C62-14-TG	0.1336±0.0337	0.1201±0.0575	0.6279	111.22
C62-15-TG	0.1013±0.0346	0.1033±0.0697	0.9513	98.08

C62-16-TG	0.0369±0.0202	0.0449±0.0355	0.6393	82.2
C62-7-TG	0.188±0.043	0.1965±0.1431	0.8931	95.67
C62-8-TG	0.1304±0.0241	0.1324±0.075	0.9526	98.5
C62-9-TG	0.0644±0.0235	0.0631±0.0166	0.908	102.1
C64-12-TG	0.0404±0.0088	0.0507±0.0158	0.196	79.74
C64-13-TG	0.0339±0.0095	0.0376±0.0125	0.5616	90.02
C64-16-TG	0.0639±0.0194	0.0901±0.052	0.2856	70.88
C64-17-TG	0.024±0.0117	0.0416±0.0295	0.2178	57.8
TG	99.7044±18.6086	89.4519±48.0029	0.6392	111.46
Total	109.5728±19.2266	95.0324±48.9411	0.5183	115.3

Annex 2: Measured compounds in muscle of eel with LC-MS techniques like Lysophosphatidyl-cholines (LPC), Phosphatidylcholine (PC), Spingomyelin (SPM) Diacylglycerols & Triacylglycerols (TG).

Compound	Migrant n=7	Pre-migrant n=6	T-test (P-value)	Change %
C16-0-LPC	0.0179±0.0067	0.0182±0.0077	0.9305	98.03
C18-0-LPC	0.0039±0.0011	0.0043±0.0007	0.4736	91.11
C18-1-LPC	0.0102±0.0062	0.0105±0.0031	0.8964	96.63
LPC	0.0319±0.0137	0.033±0.011	0.8761	96.69
C14-0-SPM	0.0424±0.0101	0.0432±0.013	0.9071	98.19
C16-0-SPM	0.0121±0.003	0.0099±0.0035	0.2598	122.16
SPM	0.0544±0.0114	0.053±0.0156	0.8593	102.65
C32-1-DG	0.0331±0.0152	0.0288±0.0101	0.5508	115.21
C32-2-DG	0.0106±0.0056	0.0103±0.0043	0.9258	102.56
C34-1-DG	0.0637±0.0268	0.0489±0.0187	0.2692	130.24
C34-2-DG	0.0409±0.0165	0.0357±0.013	0.5428	114.42
C36-1-DG	0.0127±0.0059	0.01±0.0031	0.3246	126.41
C36-2-DG	0.0384±0.0166	0.0301±0.0132	0.3359	127.57
C36-3-DG	0.0107±0.0044	0.0077±0.002	0.135	139.37
C38-2-DG	0.0052±0.0024	0.0043±0.0012	0.3846	122.48
DG	0.2153±0.0905	0.1758±0.0644	0.3799	122.48
C30-0-PC	0.0107±0.0033	0.009±0.0026	0.3261	118.64
C30-1-PC	0.0055±0.0017	0.006±0.0028	0.7622	93.1
C32-0-PC	0.0279±0.0087	0.019±0.0044	0.042	146.77
C32-1-PC	0.1357±0.0424	0.1368±0.0387	0.9613	99.18
C32-2-PC	0.0184±0.005	0.0185±0.0058	0.9503	98.96
C34-0-PC	0.0055±0.0024	0.0026±0.001	0.0183	215.92
C34-1-PC	0.8082±0.23	0.8243±0.1851	0.8917	98.05
C34-2-PC	0.1763±0.0842	0.1569±0.0331	0.5898	112.37
C34-3-PC	0.0519±0.0215	0.0327±0.0046	0.0561	158.96
C34-5-PC	0.0141±0.0056	0.017±0.0044	0.3295	83.35
C36-1-PC	0.0537±0.0151	0.0594±0.0258	0.6424	90.27

C36-2-PC	0.1334±0.0484	0.1033±0.0455	0.272	129.22
C36-4-PC	0.2815±0.1206	0.2158±0.0657	0.244	130.43
C36-5-PC	0.5945±0.2409	0.5573±0.1496	0.741	106.68
C36-6-PC	0.0529±0.0211	0.0622±0.0138	0.3591	84.95
C38-6-PC	0.8136±0.3336	0.7643±0.2679	0.7736	106.44
C38-7-PC	0.0648±0.028	0.0675±0.0144	0.8293	96.02
C40-7-PC	0.2547±0.1272	0.2414±0.13	0.8558	105.52
PC	3.5033±1.1662	3.2939±0.9139	0.7238	106.36
C32-0-PE	0.0041±0.0005	0.0036±0.0011	0.3087	114.96
C34-0-PE	0.0032±0.0011	0.0027±0.001	0.4101	118.03
C34-1-PE	0.0183±0.0028	0.0204±0.0054	0.4056	89.48
C36-1-PE	0.0535±0.014	0.059±0.0196	0.5814	90.69
PE	0.0791±0.0167	0.0857±0.0261	0.607	92.28
C16-1-ChE	0.0027±0.0007	0.0025±0.0005	0.6672	105.76
C18-0-ChE	0.0242±0.0046	0.0217±0.0041	0.3191	111.62
C18-1-ChE	0.0286±0.0073	0.0202±0.0038	0.0257*	141.32
C18-2-ChE	0.0018±0.0007	0.001±0.0003	0.038*	176.59
C18-3-ChE	0.0016±0.0015	0.0007±0.0002	0.1784	223.7
C20-4-ChE	0.0039±0.003	0.0024±0.0015	0.2722	162.94
C20-5-ChE	0.0102±0.0047	0.0075±0.0055	0.3743	135.72
C22-5-ChE	0.0116±0.0056	0.0086±0.0051	0.3248	135.57
C22-6-ChE	0.0244±0.009	0.02±0.0132	0.503	122.31
ChE	0.1089±0.0251	0.0846±0.032	0.1634	128.81
C42-0-TG	0.0195±0.0112	0.0188±0.0086	0.8918	104.08
C42-1-TG	0.0095±0.0066	0.0086±0.0041	0.7636	110.78
C44-0-TG	0.1018±0.0391	0.1211±0.0388	0.3928	84.08
C44-1-TG	0.1413±0.0713	0.1571±0.068	0.692	89.98
C44-2-TG	0.033±0.0191	0.031±0.0138	0.835	106.3
C44-3-TG	0.0094±0.0079	0.0048±0.0022	0.1849	195.12
C46-0-TG	0.2167±0.0513	0.2477±0.05	0.295	87.48
C46-1-TG	0.652±0.2272	0.7769±0.2274	0.3454	83.93
C46-2-TG	0.4178±0.201	0.4912±0.1986	0.5226	85.05
C46-3-TG	0.1181±0.0828	0.0887±0.0383	0.4231	133.13
C46-4-TG	0.0448±0.0416	0.0193±0.0089	0.1606	232.37
C46-5-TG	0.0098±0.01	0.0032±0.002	0.1309	308
C48-0-TG	0.3034±0.0683	0.3281±0.0451	0.4532	92.47
C48-1-TG	1.4157±0.3166	1.5918±0.3419	0.3599	88.94
C48-2-TG	1.5713±0.496	1.8205±0.4754	0.3758	86.31
C48-3-TG	0.7333±0.3456	0.7813±0.2876	0.7896	93.85
C48-4-TG	0.3849±0.27	0.259±0.1144	0.2932	148.61

C48-5-TG	0.1557±0.1252	0.0904±0.0417	0.2324	172.25
C48-6-TG	0.0334±0.0336	0.0126±0.0058	0.1542	266.25
C50-0-TG	0.2831±0.101	0.2962±0.0821	0.8015	95.58
C50-1-TG	2.2903±0.3893	2.407±0.4723	0.641	95.15
C50-2-TG	3.112±0.6016	3.2967±0.7325	0.6339	94.4
C50-3-TG	1.8389±0.4871	9704±0.5265	0.6517	93.32
C50-4-TG	1.1589±0.488	0.993±0.3382	0.4869	116.71
C50-5-TG	0.9807±0.5252	0.8371±0.3561	0.5717	117.16
C50-6-TG	0.3701±0.2579	0.3286±0.1494	0.7263	112.61
C52-0-TG	0.1576±0.0869	0.1844±0.089	0.5947	85.43
C52-1-TG	1.7526±0.631	1.7758±0.6747	0.9504	98.69
C52-2-TG	4.4408±0.8442	4.278±1.0994	0.774	103.81
C52-3-TG	2.5696±0.4631	2.5692±0.613	0.9989	100.02
C52-4-TG	1.4828±0.3418	1.2621±0.3236	0.2578	117.48
C52-5-TG	1.845±0.5646	1.6838±0.4944	0.5941	109.57
C52-6-TG	1.7511±0.7424	1.787±0.6318	0.9267	97.99
C52-7-TG	0.6073±0.4053	0.6837±0.3028	0.7053	88.82
C52-8-TG	0.1881±0.1592	0.0957±0.0447	0.1843	196.65
C52-9-TG	0.0529±0.0511	0.0205±0.0103	0.1479	258.19
C54-1-TG	0.6088±0.3257	0.6448±0.3478	0.8515	94.41
C54-10-TG	0.0949±0.0738	0.0531±0.0252	0.1995	178.75
C54-2-TG	2.381±0.8129	2.3865±0.994	0.9916	99.77
C54-3-TG	2.514±0.5653	2.4093±0.7661	0.7884	104.35
C54-4-TG	1.0203±0.2657	0.8924±0.1997	0.3444	114.33
C54-5-TG	1.5161±0.2834	1.3543±0.3066	0.3485	111.94
C54-6-TG	2.447±0.663	2.4336±0.5779	0.9696	100.55
C54-7-TG	1.755±0.6399	1.8962±0.552	0.6777	92.56
C54-8-TG	0.7704±0.3927	0.6976±0.2763	0.7041	110.43
C54-9-TG	0.3263±0.2333	0.2039±0.0926	0.2374	160.04
C56-1-TG	0.0507±0.0298	0.0589±0.0347	0.6639	86.22
C56-10-TG	0.4516±0.2444	0.3788±0.1696	0.5416	119.22
C56-11-TG	0.1435±0.0883	0.1134±0.0533	0.4681	126.49
C56-2-TG	0.487±0.2663	0.5448±0.3098	0.7282	89.39
C56-3-TG	0.9128±0.364	0.967±0.4161	0.8092	94.4
C56-4-TG	0.41±0.1273	0.3909±0.1084	0.7758	104.88
C56-5-TG	0.7039±0.1344	0.6728±0.1335	0.6847	104.62
C56-6-TG	1.3534±0.3937	1.4507±0.2678	0.6091	93.29
C56-7-TG	1.6686±0.4176	1.7646±0.3963	0.6794	94.56
C56-8-TG	0.9195±0.2809	0.9446±0.2349	0.8638	97.34

C56-9-TG	0.5921±0.2563	0.5127±0.1861	0.5328	115.47
C58-10-TG	0.5284±0.1876	0.5123±0.1485	0.8662	103.14
C58-11-TG	0.5053±0.236	0.517±0.1949	0.9235	97.73
C58-12-TG	0.1584±0.0843	0.1586±0.0717	0.9955	99.84
C58-13-TG	0.0269±0.0193	0.0178±0.0079	0.2846	151.52
C58-2-TG	0.0581±0.0354	0.0712±0.042	0.5604	81.62
C58-3-TG	0.1666±0.0939	0.1963±0.111	0.6172	84.87
C58-4-TG	0.2263±0.1047	0.242±0.1071	0.7957	93.53
C58-5-TG	0.3216±0.0915	0.3117±0.0992	0.8552	103.2
C58-6-TG	0.5154±0.1343	0.5184±0.1206	0.9679	99.44
C58-7-TG	0.8539±0.192	0.8839±0.1754	0.7743	96.61
C58-8-TG	0.6279±0.1244	0.6377±0.1313	0.8941	98.47
C58-9-TG	0.4087±0.1098	0.3697±0.0913	0.4981	110.57
C60-10-TG	0.2675±0.0642	0.2689±0.0539	0.9653	99.46
C60-11-TG	0.4376±0.1488	0.4337±0.182	0.9677	100.89
C60-12-TG	0.3745±0.1597	0.4134±0.1183	0.6249	90.59
C60-13-TG	0.1047±0.054	0.1023±0.0572	0.9419	102.26
C60-14-TG	0.0293±0.0196	0.0195±0.0091	0.2676	150.31
C60-15-TG	0.01±0.007	0.007±0.0038	0.3463	143.29
C60-2-TG	0.0078±0.0041	0.0085±0.0047	0.7897	92.06
C60-3-TG	0.024±0.013	0.0262±0.0146	0.7841	91.69
C60-4-TG	0.0336±0.0179	0.0375±0.0202	0.7207	89.56
C60-5-TG	0.0908±0.0388	0.0925±0.0454	0.9447	98.18
C60-6-TG	0.1752±0.067	0.1821±0.0756	0.8664	96.22
C60-7-TG	0.2731±0.0944	0.2962±0.0945	0.67	92.23
C60-8-TG	0.202±0.0434	0.2134±0.0384	0.6228	94.62
C60-9-TG	0.1168±0.0225	0.1086±0.0188	0.4887	107.55
C62-10-TG	0.0806±0.0199	0.0776±0.0109	0.7405	103.84
C62-12-TG	0.2106±0.0703	0.2339±0.0431	0.4808	90.03
C62-13-TG	0.132±0.0503	0.1384±0.0347	0.7916	95.36
C62-14-TG	0.0477±0.0227	0.0425±0.0156	0.6382	112.15
C62-15-TG	0.0349±0.0191	0.0283±0.0128	0.4745	123.35
C62-16-TG	0.0127±0.0076	0.0104±0.0051	0.5244	122.61
C62-7-TG	0.0924±0.0407	0.1028±0.0481	0.6865	89.91
C62-8-TG	0.0777±0.0257	0.0846±0.0279	0.6545	91.83
C62-9-TG	0.0404±0.0078	0.0428±0.009	0.6264	94.47
C64-12-TG	0.0399±0.016	0.046±0.0092	0.4072	86.62
C64-13-TG	0.0267±0.0081	0.03±0.0054	0.3919	88.79
C64-16-TG	0.0267±0.014	0.0273±0.0111	0.9361	97.91
C64-17-TG	0.0094±0.0052	0.0094±0.0044	0.9758	99.13

TG	59.7561±16.9317	59.6124±17.3311	0.9883	100.24
Total	63.7491±17.7421	63.3384±18.2405	0.9681	100.65

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