

Tissue-specific Biomarker Metabolites of Meat, Fat and Egg of Siberian Sturgeon

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Abstract Metabolites in the edible tissues of meat, fat, and egg from a female Siberian sturgeon aged four years were analyzed by non-targeted metabolomics based on ultra-performance liquid chromatography (UPLC) and tandem mass spectrometry (MS-MS). A total of 383 metabolites were detected, and 248 were assigned (annotated) to metabolite classes. The major shared metabolites among the edible tissues were a kaurenoic acid-derivative, oleic acid, choline, and L-carnitine. Although all 383 metabolites were present in all the tissues, their respective metabolomic profiles were separated by statistical analyses. A discriminant analysis screened tissue-specific biomarker metabolites, such as nicotinamide (a vitamin B3 vitamer) and taurine in meat; allopurinol (a structural isomer of hypoxanthine), carnosine, inosine 5'-phosphate (inosine monophosphate, IMP), and L-(+)-lactic acid in fat; and N,N-dimethylsphingosine, 3-(2-hydroxyphenyl)propanate, L-glutamic acid, and docosahexaenoic acid (DHA) in egg. The biomarker metabolites were projected on metabolic maps to predict relevant metabolic pathways. Lipid and amino acid metabolisms were the major assigned (annotated) pathways, though not-assigned (not-annotated, NA) pathways were the most dominant. Integration of metabolomic profiles with multiple approaches and platforms will contribute to enhancing the utilization of processed byproducts of sturgeons and improving the breeding and husbandry of sturgeon stocks.

Keywords: Non-targeted metabolomics, *Acipenser baerii*, discriminant analysis, metabolic pathways

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1. Introduction

The Siberian sturgeon (*Acipenser baerii* Brandt, 1869) is a freshwater fish belonging to the family *Acipenseridae*, widely distributed from subtropical to subarctic rivers, lakes, and coastlines of Eurasia and North America [1]. Sturgeons are one of the oldest surviving vertebrates on the earth and are thought to have evolved during the Jurassic period about 200 million years ago [2]. Known as a living fossil in water, it is a vital fishery resource in North America and Eurasia. Sturgeon meat is a delicious ingredient that can provide humans with abundant nutrients such as high-quality protein, amino acids, and unsaturated fatty acids. The caviar made from sturgeon roe is known as "black gold." Due to its high nutritional and economic value, sturgeon has become an essential economic fish globally. However, under the influence of human activities, almost all species of sturgeon are highly threatened or even face the risk of extinction due to habitat destruction, overfishing, environmental pollution, and dam construction [3-5]. Since 1997, sturgeons have been protected by the Washington Convention. As a result, the demand for sturgeon aquaculture is becoming increasingly important [6].

The growth and sexual maturity of sturgeon are slow [7]. It takes several years or more from hatching sturgeon to produce caviar and meat against the expanding demands [8,9]. The continuous improvement of artificial hatchery technology, breeding environment, and feed for sturgeon has recently accelerated the sexual maturity and growth rate of sturgeon [10,11]. However, basic research on the metabolomics of sturgeon has yet to be carried out so far. Due to high chromosome counts and chromosome aneuploidy of sturgeon [1,12,13], genetic engineering and genome-based metabolic engineering are rather tricky, while metabolomics would be promising.

Genomics, proteomics, and metabolomics are the three most frequently used techniques in nutrition and physiology-based 'omics' research aimed at comprehending and characterizing numerous biological processes related to nutrition and diet [14]. In biological research, metabolomics is widely employed. After genomics and proteomics, metabolomics is a relatively recent scientific field. Many cellular life processes occur at the metabolite level, and metabolites are the end products of gene expression. Genomics and proteomics investigate life activities from the gene and protein levels, respectively [15]. Metabolomics studies the relationship between metabolites and physiological changes via qualitative and quantitative analysis of all low-molecular-mass

metabolites (relative molecular mass less than 1000) genuinely present in an organism [16,17]. The comprehensive overview of metabolites makes it possible to identify the characteristic metabolites and metabolic pathways in Siberian sturgeon without interfering with the genomic diversity and complexity.

Previous studies have reported Siberian sturgeon's aqueous metabolite profile [18] and lipidomic profile [19]. As a follow-up, this study presents the metabolomic profile of non-targeted metabolites in three different tissues (meat, fat, and egg) of Siberian sturgeon, identifies tissue-specific biomarker metabolites, and provides the theoretical basis of metabolism for the aquaculture of Siberian sturgeon.

2. Materials and Methods

2.1. Siberian Sturgeon and Tissues Samples

A female Siberian sturgeon (*Acipenser baerii*) aged four years and weighing 3.5 kilograms was the same specimen reported for aqueous and lipophilic metabolites [18,19]. Dry pellets composed of fishmeal, flour, soybean oilcake, and maize gluten meal were fed to the animal. The fish was dissected on-site, and samples of edible tissues, i.e., meat, fat, and egg, were stored initially at -20°C and later within the same day at -70°C until analysis.

Three sub-samples from each tissue, i.e., a total of nine samples, were collected on dry ice in the university laboratory and shipped with dry ice to the laboratory of the BGI (formerly Beijing Genomics Institute; Shenzhen, Guangdong, China) having expertise in non-targeted metabolomics [20].

2.2. Non-targeted metabolomic Analysis

2.2.1. Sample Preparation

At the BGI laboratory, a 25-mg tissue from each sub-sample was weighed and placed in a centrifuge tube. Simultaneously added were: two magnetic beads; a 10- μ l internal standard; and an 800- μ l pre-cooled extraction reagent (methanol: acetonitrile: water, 2:2:1, v/v/v). The cocktail was ground at 50 Hz grind for 5 min, placed at -20°C for 2 h, and centrifuged at 25,000 g at 4°C for 15 min. A 600- μ l supernatant was transferred to another centrifuge tube, lyophilized, re-suspended in the added 600- μ l 50% methanol, shaken to complete dissolution, and centrifuged again at 25,000 g at 4°C for 15 min. The final supernatant was transferred to a final centrifuge tube, from which a 10- μ l aliquot was used for the following LC-MS/MS analysis.

2.2.2. Liquid Chromatography (LC)

Ultra-performance liquid chromatographic separation of metabolites was performed on an ACQUITY UPLC I-Class PLUS using an ACQUITY UPLC BEH C18 column (Waters, Milford, Massachusetts, USA) at the column temperature of 45°C. The mobile phase consisted of: 0.1% formic acid and acetonitrile (ACN) in the positive mode; and, 10mM ammonium formate and ACN in the negative mode. The gradient conditions were as follows: 0-1 min,

2% ACN; 1-9 min, 2% to 98% ACN; 9-12 min, 98% ACN; 12-12.1 min, 98% to 2% ACN; and 12.1-15min, 2% ACN. The flow rate was 0.35 ml/min, and the injection volume was 5 μ l.

2.2.3. Tandem Mass Spectrometry (MS/MS)

Primary and secondary mass spectrometry analyses were performed using a tandem of Orbitrap Q Exactive mass spectrometers (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The full scan range was 70–1050 m/z with a resolution of 70,000, and the automatic gain control (AGC) target for MS acquisitions was set to 3×10^6 with a maximum ion injection time of 100 ms. The top three precursors were selected for subsequent fragmentation with a maximum ion injection time of 50 ms and resolution of 17500; the AGC was set to 1×10^5 . The stepped normalized collision energy was set to 20, 40, and 60 eV. Parameters of electrospray ionization were set as follows: the sheath gas flow rate, 40 units (0.75 l/min); the auxiliary gas flow rate, 10 units (6 l/min); positive-ion mode spray voltage (kV), 3.80; negative-ion mode spray voltage (kV), 3.20; the capillary temperature, 320°C; and the auxiliary gas heater temperature, 350°C.

2.2.4. Data Acquisition and Pre-processing

The MS/MS raw data were imported offline into the software Compound Discoverer 3.3 (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and analyzed online with the MS databases of BGI metabolome database (BMDB), mzCloud (<https://www.mzcloud.org/>), and ChemSpider (<http://www.chemspider.com/>). A data matrix containing metabolite peak areas and identification results was thus obtained and tabularized for bioinformatic analyses.

2.3. Statistical Analyses

2.3.1. Multivariate Statistical Analysis

Metabolite data were log-transformed to improve their normality before statistical analysis. To visualize the relatedness of the metabolomic profiles of the meat, fat, and egg tissues, principal component analysis (PCA) and hierarchical clustering analysis (HCA) were performed online at the OmicStudio, LC-Bio Technologies Co. Ltd. (<https://www.omicstudio.cn>).

2.3.2. Bioinformatic Analysis

Linear discriminant analysis (LDA) effect size analysis (LEfSe) [21], an analytical tool for screening differential metabolites as biomarkers among high-dimensional datasets, was performed online at the Huttenhower Lab, Biostatistics Department, Harvard T. H. Chan School of Public Health (<https://huttenhower.sph.harvard.edu/lefse/>). It can realize the comparison between two or more groups and can find out the biomarkers between groups.

Differential metabolites were further projected on the metabolic pathway maps generated by the Kyoto Encyclopedia of Genes and Genomes (KEGG; <https://www.genome.jp/kegg/pathway.html>) [22,23]. The pathways are divided into three levels: the first-level (super pathways), second-level (pathways), and third-level (sub-pathways).

3. Results

3.1. Non-targeted Metabolomic Profiles of Meat, Fat, and Egg of a Siberian Sturgeon

Non-targeted metabolomics is an unbiased, comprehensive, and systematic analysis of endogenous metabolites in organisms, through which tissue-specific metabolites or biomarkers can be identified. A total of 383 metabolites were detected in the studied Siberian sturgeon's meat, fat, and egg tissues (Table S1; https://www.sturgeon-metabolome.hiroshima-u.ac.jp/h0888_Siberia_meat-fat-egg.ods), of which 248 were assigned (annotated) to metabolite classes (super-classes, classes, and sub-classes) defined in the Human Metabolome Database (HMDB; <https://hmdb.ca/metabolite>) [24] and the rest 135 were detected but not assigned (not annotated, "NA"). The most dominant metabolite in meat and fat was an "NA" of $C_{20}H_{30}O_2$ (*1), probably derived from kaurenoic acid (*2) which is known for antioxidant and antimicrobial properties [25]. In egg, the $C_{20}H_{30}O_2$ "NA" followed *N,N*-dimethylsphingosine, which is an inhibitor of sphingosine kinase dually functioning as the extra-/intra-cellular lipid signaling molecule [26].

*1: 5,9-dimethyl-14-methylidene tetracyclohexadecane-5-carboxylic acid ($C_{20}H_{30}O_2$)

*2: 5,9-dimethyl-14-methylene tetracyclohexadecane-5-carboxylic acid ($C_{20}H_{30}O_2$)

Oleic acid (an 18:1 fatty acid), choline, L-carnitine, and inosine 5'-monophosphate were commonly abundant in all the tissues, followed by L-lactic acid, alanine, and creatine which were regarded as major metabolites in the previous study focusing on aqueous metabolites [18]. Less aqueous, more hydrophobic (oleophilic) ones were detected in this study, which resulted in different metabolomic profiles, as discussed later in the Discussion section.

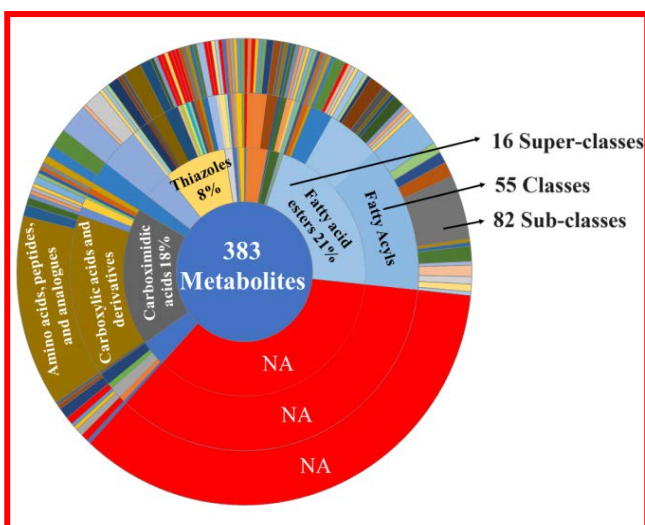


Figure 1. Sunburst chart to visualize the statistics and hierarchical assignment (annotation) of the detected 383 metabolites in a Siberian sturgeon. The innermost, intermediate, and outermost rings represent the super-class, class, and sub-class categories, respectively, based on the Human Metabolome Database (HMDB; <https://hmdb.ca/metabolite>) [24]. "NA" represents "not assigned" (not annotated). A total of 135, 136, and 150 "NAs" were excluded from the counts of super-class, class, and sub-class counts, respectively.

The breakdown of the 248 assigned metabolites is as follows.: 56 fatty acyls; 37 amino acids, peptides, and analogs; 18 organic acids; 18 organoheterocyclic compounds; 15 proteinogenic amino acids; 14 steroids and derivatives; 11 purines and derivatives; 9 carbohydrates; 7 nucleic acids and analogs; 7 terpenoids; 6 benzene and derivatives; 5 organic oxygen compounds; 5 amines; (Figure 1; Table S2).

3.2. Multivariate Statistical Analyses

Principal component analysis (PCA) is a method for the statistical analysis of multidimensional data with unsupervised pattern recognition, which aims to reveal the internal structure of multiple variables through a small number of principal components by using dimensionality reduction methods. PCA analysis is used to reflect the characteristics of metabolomics under multidimensional data through several principal components. Therefore, through the PCA analysis, we can observe the dispersion of replications within the group and the differences between different groups. In this study, the results of PCA analysis showed significant separation among different tissue samples of Siberian sturgeon, which indicated that there were significant changes and differences in metabolites in different tissue samples of Siberian sturgeon. In the PCA diagram (Figure 2), the first principal component (PC1) explained 68.6% of the total variation in the original dataset; the second principal component (PC2) explained 22.6% of the total variation in the original dataset. The PCA diagram also showed that the replications within the groups are highly cohesive or even partially overlapped, indicating that the dispersion within the groups is extremely low and the data reliability is extremely high.

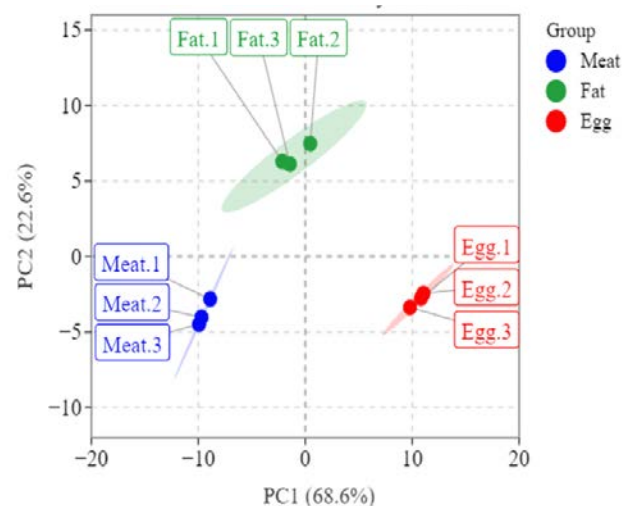


Figure 2. PCA plot of non-targeted metabolites in meat, fat, and eggs of Siberian sturgeon. High cohesion within tissue groups and significant separation among the three different tissues of the studied Siberian sturgeon

Significant separation among the three tissues, i.e., meat, fat, and egg, was confirmed by the hierarchical clustering analysis on the tissue-derived metabolites (Figure 3). Samples of the same tissue were grouped in the same cluster, while the samples of different tissues were separated from each other.

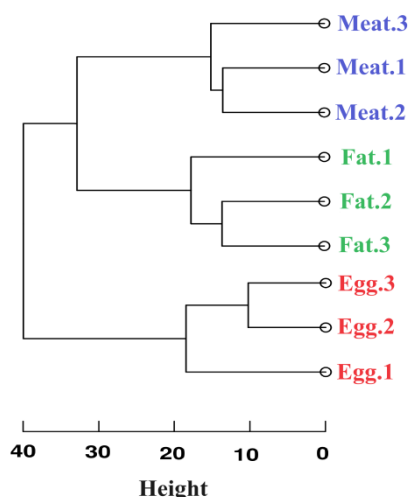


Figure 3. Dendrogram based on the hierarchical clustering analysis on metabolite profiles of the samples from meat, fat, and egg tissues of a Siberian sturgeon

3.3. Bioinformatic Analyses

3.3.1. LEfSe Biomarker Analysis

The metabolomic profiles of the meat, fat, and egg tissues of a Siberian sturgeon were screened for differential metabolites as biomarkers using the linear discriminant analysis (LDA) Effect Size (LEfSe). A total of 46 biomarker metabolites were screened with LDA scores >3 , of which 13, 12, and 21 were from meat, fat, and egg, respectively (Figure 4 and Table S3). Among them, the top biomarkers with the LDA scores >4 (shown in red in Table S2) were:

- for meat, nicotinamide (a vitamin B₃ vitamer), and taurine;
- for fat, allopurinol (a structural isomer of hypoxanthine), carnosine, inosine 5'-phosphate (inosine monophosphate, IMP), and L-(+)-lactic acid; and,
- for egg, *N,N*-dimethylsphingosine which is an inhibitor of sphingosine kinase [26], 3-(2-hydroxyphenyl)propanate, L-glutamic acid, and docosahexaenoic acid (DHA).

3.3.2. KEGG Pathway Analysis

A total of 46 overall (not issue-specific) biomarker metabolites with LDA scores >3 (Figure 4, Table S3) were projected on the KEGG metabolic pathway maps, excluding the “Human Diseases” category, to predict relevant metabolisms. The pathways are divided into levels 1, 2, and 3, corresponding to super-pathways, pathways, and sub-pathways, respectively. Statistics of the predicted pathways at levels 1 and 2 are shown in Figure 5 for overall biomarker metabolites and in Figures S1 to S3 for tissue-specific biomarker metabolites. The sum of the metabolite numbers, 72 and 119 at levels 1 and 2, respectively (Figure 5), exceed the number of screened biomarker metabolites because some metabolites may be involved in more than one category. For example, L-glutamic acid, an egg biomarker (Figure 4, Table S3), was involved in five super-pathways at level 1, 19 pathways at level 2, and 43 sub-pathways at level 3 (Table S4). In

contrast, docosahexaenoic acid (DHA), also an egg biomarker, was involved in one super-pathway, one pathway, and one sub-pathway at all the levels of 1, 2, and 3, respectively (Table S4).

At level 1, one-third or *ca.* 33% of the biomarker metabolites were involved in the “Metabolism” super-pathway, followed by the “NA” (not assigned, not annotated) category. Among other super-pathways, “Environmental information processing”, “Organismal systems”, “Cellular processes”, and “Genetic information processes” appeared to involve the biomarker metabolites (Figure 5). At level 2, the “NA” and the vast “Global and overview maps” were the dominant pathways, involving *ca.* 18% and 17%, respectively, of the biomarker metabolites. More specific pathways “Amino acid metabolism” and “Metabolism of other amino acids” followed, the latter of which consisted of the level 3 sub-pathways “beta-Alanine metabolism”, “D-Amino acid metabolism”, “Taurine and hypotaurine metabolism”, and “Glutathione metabolism”. Sub-pathways at level 3, not depicted but listed in Table S4, provide insights into detailed metabolisms and would be implicative of improved production of sturgeons [27].

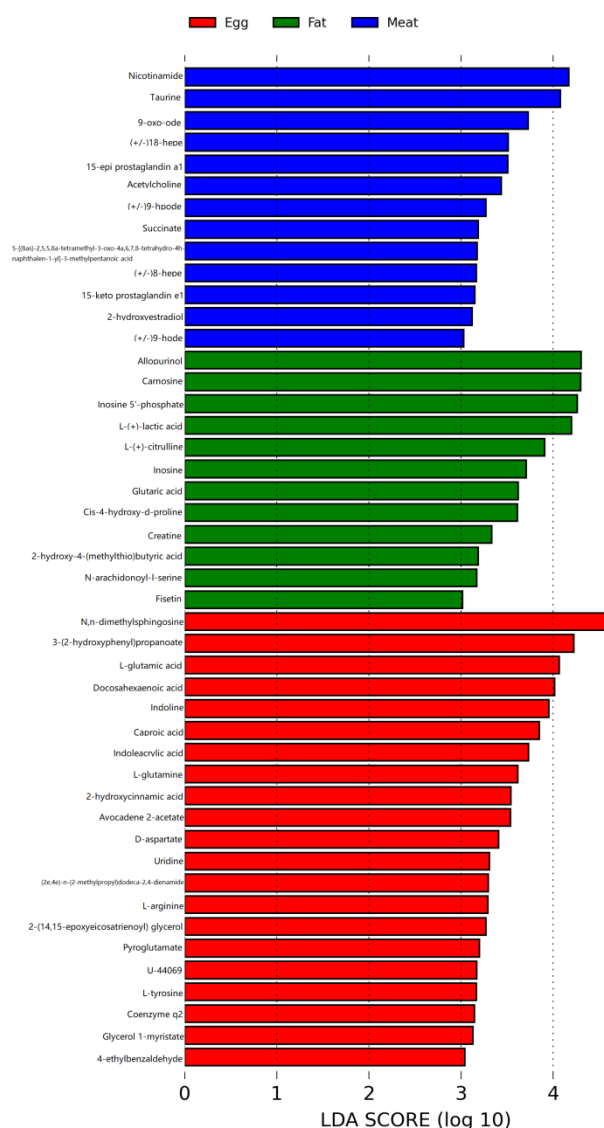


Figure 4. Differential biomarker metabolites with LDA scores >3 in meat, fat, and egg tissues of a Siberian sturgeon

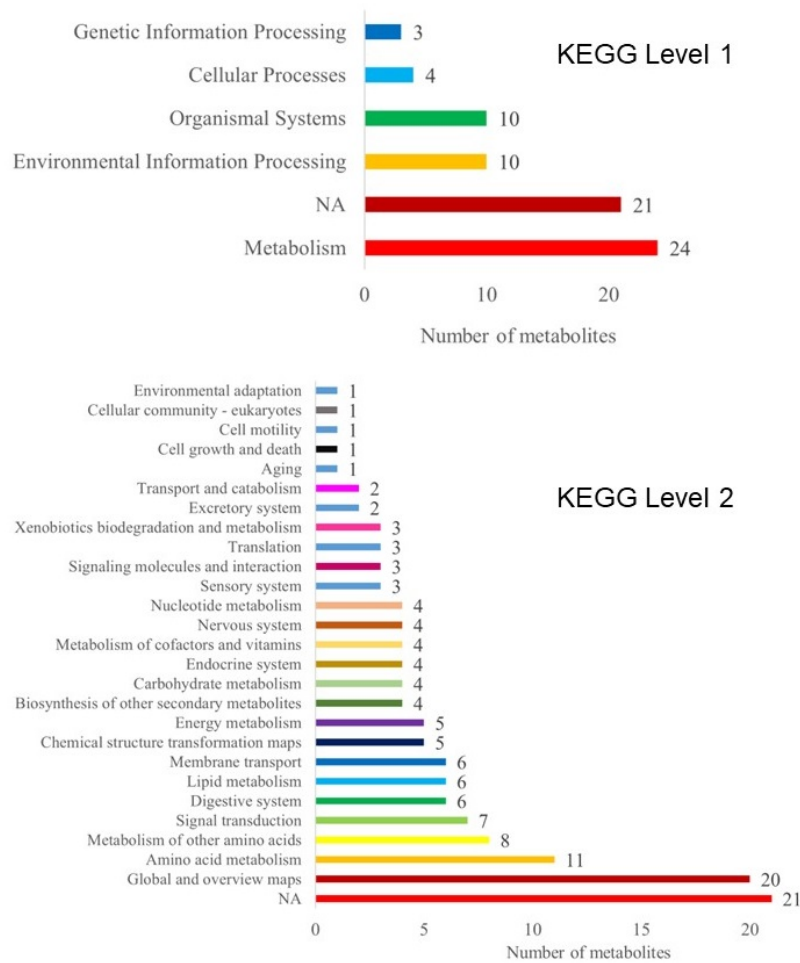


Figure 5. Statistics of KEGG super-pathways and pathways predicted at levels 1 and 2, respectively, for the overall (not tissue-specific) detected metabolites (<https://www.genome.jp/kegg/pathway.html>) [22,23]. The X-axis represents the number of metabolites involved in a super-pathway/pathway. The Y-axis shows the KEGG super-pathway and pathways, from which the category “Human Diseases” is excluded. NA, not assigned (not annotated)

4. Discussion

The non-targeted metabolomics in this study is based on ultra-performance liquid chromatography and tandem mass spectrometry (UPLC-MS-MS) at BGI, resulting in the detection of 383 molecular species of metabolites. The 383 metabolites were shared by all the meat, fat, and egg tissues, making a Venn diagram unnecessary. In contrast, relatively a small number of molecular species, 245, were detected by a targeted metabolomics based on capillary electrophoresis and time-of-flight mass spectrometry (CE-TOFMS) at Human Metabolome Technologies (HMT) [18]. Of the overall metabolites, only 74 were found in both non-targeted and targeted metabolomic profiles as shown by the Venn diagram (Figure 6; tissue-specific Venn diagrams are shown in Figure S4). The same tendency of 1) more metabolites detected by the non-targeted approach, 2) fewer metabolites detected by the targeted approach, and 3) relatively a small number of metabolites commonly detected by both approaches was also reported in a previous study [28].

The 309 metabolites detected only in non-targeted (BGI \ HMT), 171 only in targeted (HMT \ BGI), and 74 in both sets (BGI $\cap$ HMT) are listed in Table S5. All the 383 metabolite species detected by the non-targeted approach

were present in all the tissues, i.e., meat, fat, and egg, while only part of the 245 targeted metabolites were seen in each tissue (Figure S4). The difference in the non-targeted and targeted metabolites may be due to the difference in the analytical platforms, i.e., non-targeted UPLC-MS-MS at BGI and targeted CE-TOFMS at HMT. Previous studies reported that both targeted and non-targeted approaches led to similar conclusions or directions [29,30].

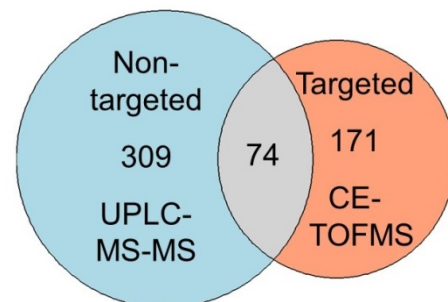


Figure 6. Distribution of the 383 and 245 metabolites of a Siberian sturgeon, detected by the non-targeted UPLC-MS-MS at BGI and the targeted CE-TOFMS at HMT, respectively. Relatively a small number of metabolites, 74, were commonly detected by both approaches/platforms. Tissue-specific Venn diagrams are shown in Figure S4

The numbers and the species of detectable metabolites differ among 1) the separation techniques such as UPLC and CE, 2) the detection platforms such as mass spectrometry (MS-MS and TOFMS) and nuclear magnetic resonance (NMR) spectroscopy, and, 3) their combinations. Although NMR platforms have been used in sturgeon metabolomics [31-37], a human-targeted study suggested that CE-MS has advantages over NMR in terms of reduced sample volume, reduced operational costs, and increased metabolome coverage [38]. Metabolomic profiling would be more integrative by the use of multiple separation techniques and detection platforms as shown by previous studies [39,40] as well as our previous [18,19] and current studies.

Biomarker metabolites of the studied sturgeon tissues were screened and projected onto KEGG maps to predict relevant metabolic pathways. Insights from the screened metabolites and the predicted metabolisms would contribute to the more effective utilization of processed byproducts of sturgeons [41], as well as to improvements in the breeding and husbandry of sturgeon stocks.

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Statement of Competing Interests

The authors declare that there is no conflict of interest.

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Supplementary materials

Contents

Table S1. List of a total of 383 metabolites identified in a Siberian sturgeon (separate sheet available online at https://www.sturgeon-metabolome.hiroshima-u.ac.jp/h0888_Siberia_meat-fat-egg.ods).

Table S2. Statistics of the annotated metabolites at the super-class and class levels.

Table S3. Tissue-specific biomarker metabolites having LDA scores >3 screened by LEfSe.

Table S4. Lists of the KEGG pathways predicted at levels 1, 2, and 3 for the biomarker metabolites with LDA scores >3 in Table S3 (separate sheet available online at https://www.sturgeon-metabolome.hiroshima-u.ac.jp/h0888_Table_S4_KEGG.ods).

Table S5. Lists of the metabolites detected only in the non-targeted set, only in the targeted set, and in both sets (separate sheet available online at https://www.sturgeon-metabolome.hiroshima-u.ac.jp/h0888_Table_S5_Venn.ods).

Figure S1. Tissue-specific (meat-specific) KEGG metabolic pathways at levels 1 and 2.

Figure S2. Tissue-specific (fat-specific) KEGG metabolic pathways at levels 1 and 2.

Figure S3. Tissue-specific (egg-specific) KEGG metabolic pathways at levels 1 and 2.

Figure S4. Distribution of the metabolites in meat (topmost), fat (middle), and egg (bottommost).

Table S1. List of a total of 383 metabolites identified in meat, fat, and egg tissues of a Siberian sturgeon, of which 135 were identified but not assigned to metabolite classes (super-classes, classes, and sub-classes) defined in the Human Metabolome Database (HMDB; <https://hmdb.ca/metabolite>) [24]. The downloadable table is available online at the following URL: https://www.sturgeon-metabolome.hiroshima-u.ac.jp/h0888_Siberia_meat-fat-egg.ods.

Table S2. Statistics of the metabolites detected by BGI and their assignments to the super-classes and classes categorized by the Human Metabolome Database (HMDB; <https://hmdb.ca/metabolite>) [24]. *1 A total of 135 not-assigned (NA) metabolites were excluded from the superclass counts. *2 A total of 136 (135+1) not-assigned (NA) metabolites were excluded from the class counts.

Super-class	Number of metabolites	% of total	Class	Number of metabolites	% of total
Not-assigned (NA) (*1)	135	35.25%	NA (*2)	135	35.25%
Alkaloids and derivatives	1	0.26%	NA (*2)	1	0.26%
Benzenoids	11	2.87%	Benzene and substituted derivatives	7	1.83%
			Phenols	4	1.04%
Carbohydrates	1	0.26%	Monosaccharides	1	0.26%
FA Fatty acyls	5	1.31%	FA02 Octadecanoids	2	0.52%
			FA03 Eicosanoids	2	0.52%
			FA08 Fatty amides	1	0.26%
Flavonoids	1	0.26%	Flavonoids	1	0.26%
Homogeneous non-metal compounds	1	0.26%	Non-metal oxoanionic compounds	1	0.26%
Lipids and lipid-like molecules	82	21.41%	Endocannabinoids	1	0.26%
			Glycerolipids	2	0.52%
			Glycerophospholipids	1	0.26%
			Prenol lipids	8	2.09%
			Steroids and steroid derivatives	19	4.96%
			Fatty Acyls	51	13.32%
Nucleosides, nucleotides, and analogs	13	3.39%	5'-deoxyribonucleosides	1	0.26%
			Purine nucleosides	2	0.52%
			Purine nucleotides	4	1.04%
			Pyrimidine nucleosides	2	0.52%
			Pyrimidine nucleotides	4	1.04%
Organic acids and derivatives	69	18.02%	Carboximidic acids and derivatives	1	0.26%
			Carboxylic acids and derivatives	56	14.62%
			Hydroxy acids and derivatives	3	0.78%
			Keto acids and derivatives	3	0.78%
			Organic carbonic acids and derivatives	1	0.26%
			Organic phosphoric acids and derivatives	1	0.26%
			Organic sulfonic acids and derivatives	2	0.52%
			Peptidomimetics	2	0.52%
Organic nitrogen compounds	8	2.09%	Organonitrogen compounds	8	2.09%
Organic oxygen compounds	14	3.66%	Organooxygen compounds	14	3.66%
Organoheterocyclic compounds	32	8.36%	Azoles	1	0.26%
			Benzazepines	1	0.26%
			Benzopyrans	1	0.26%
			Diazines	3	0.78%
			Dithiolanes	1	0.26%
			Furans	1	0.26%
			Imidazopyrimidines	5	1.31%
			Indoles and derivatives	5	1.31%
			Isobenzofurans	1	0.26%
			Isoquinolines and derivatives	1	0.26%
			Lactones	1	0.26%
			Naphthofurans	1	0.26%
			Oxanes	1	0.26%
			Piperidines	1	0.26%
			Pteridines and derivatives	2	0.52%
			Pyrazolopyrimidines	1	0.26%
			Pyridines and derivatives	2	0.52%
			Quinolines and derivatives	3	0.78%
Phenylpropanoids and polyketides	4	1.04%	Phenylpropanoic acids	1	0.26%
			Cinnamic acids and derivatives	2	0.52%
			Coumarins and derivatives	1	0.26%
PK Polyketides	2	0.52%	PK13 Aromatic polyketides	1	0.26%
			PK12 Flavonoids	1	0.26%
ST Sterol Lipids	1	0.26%	ST04 Bile acids and derivatives	1	0.26%
Terpenoids	3	0.78%	Sesquiterpenoids (C15)	2	0.52%
			Diterpenoids (C20)	1	0.26%
Total 16 super-classes (*1)	383	100%	Total 55 classes (*2)	383	100%

Table S3. Tissue-specific biomarker metabolites having LDA scores >3 screened by LEfSe. Biomarkers with the LDA scores >4 are shown in red.

Table S4. Lists of the KEGG pathways predicted from the biomarker metabolites with LDA scores >3 in Table S3 (separate sheet available online at https://www.sturgeon-metabolome.hiroshima-u.ac.jp/h0888_Table S4_KEGG.ods).

Table S5. Lists of the metabolites detected only in the non-targeted set, only in the targeted set, and in both sets (separate sheet available online at https://www.sturgeon-metabolome.hiroshima-u.ac.jp/h0888_Table S5_Venn.ods)

Tissue	Metabolite	LDA score
Meat (13)	Nicotinamide	4.172
	Taurine	4.081
	(10E,12Z)-9-oxooctadeca-10,12-dienoic acid	3.731
	(5Z,8Z,11Z,14Z,16E)-18-hydroxyicosa-5,8,11,14,16-pentaenoic acid	3.514
	7-[2-(3-hydroxy oct-1-enyl)-5-oxocyclopent-3-en-1-yl]heptanoic acid	3.509
	Acetylcholine	3.437
	(10E,12Z)-9-hydroperoxyoctadeca-10,12-dienoic acid	3.270
	Succinate	3.187
	5-[(8aS)-2,5,5,8a-tetramethyl-3-oxo-4a,6,7,8-tetrahydro-4H-naphthalen-1-yl]-3-methylpentanoic acid	3.175
	(5Z,9E,11Z,14Z,17Z)-8-hydroxyicosa-5,9,11,14,17-pentaenoic acid	3.166
	7-[(1R,2R,3R)-3-hydroxy-5-oxo-2-[(E)-3-oxooct-1-enyl]cyclopentyl]heptanoic acid	3.149
	2-hydroxyestradiol	3.119
	9-hydroxyoctadeca-10,12-dienoic acid	3.028
Fat (12)	Allopurinol	4.303
	Carnosine	4.302
	Inosine 5'-phosphate	4.261
	L-(+)-lactic acid	4.199
	L-(+)-citrulline	3.910
	Inosine	3.710
	Glutaric acid	3.621
	Cis-4-hydroxy-d-proline	3.612
	Creatine	3.334
	2-hydroxy-4-(methylthio)butyric acid	3.188
	N-arachidonoyl-l-serine	3.171
Egg (21)	Fisetin	3.019
	N,N-dimethylsphingosine	4.612
	3-(2-hydroxyphenyl)propanoate	4.225
	L-glutamic acid	4.068
	Docosahexaenoic acid	4.017
	Indoline	3.953
	Caproic acid	3.850
	Indoleacrylic acid	3.732
	L-glutamine	3.616
	2-hydroxycinnamic acid	3.543
	Avocadene 2-acetate	3.539
	D-aspartate	3.409
	Uridine	3.309
	(2E,4E)-N-(2-methylpropyl)dodeca-2,4-dienamide	3.297
	L-arginine	3.292
	2-(14,15-epoxyeicosatrienoyl) glycerol	3.270
	Pyroglutamate	3.200
	(Z)-7-[(1S,4R,5S,6R)-5-[(E,3S)-3-hydroxyoct-1-enyl]-2-oxabicyclo[2.2.1]heptan-6-yl]hept-5-enoic acid	3.170
	L-tyrosine	3.168
	Coenzyme q2	3.147
	Glycerol 1-myristate	3.131
	4-ethylbenzaldehyde	3.041

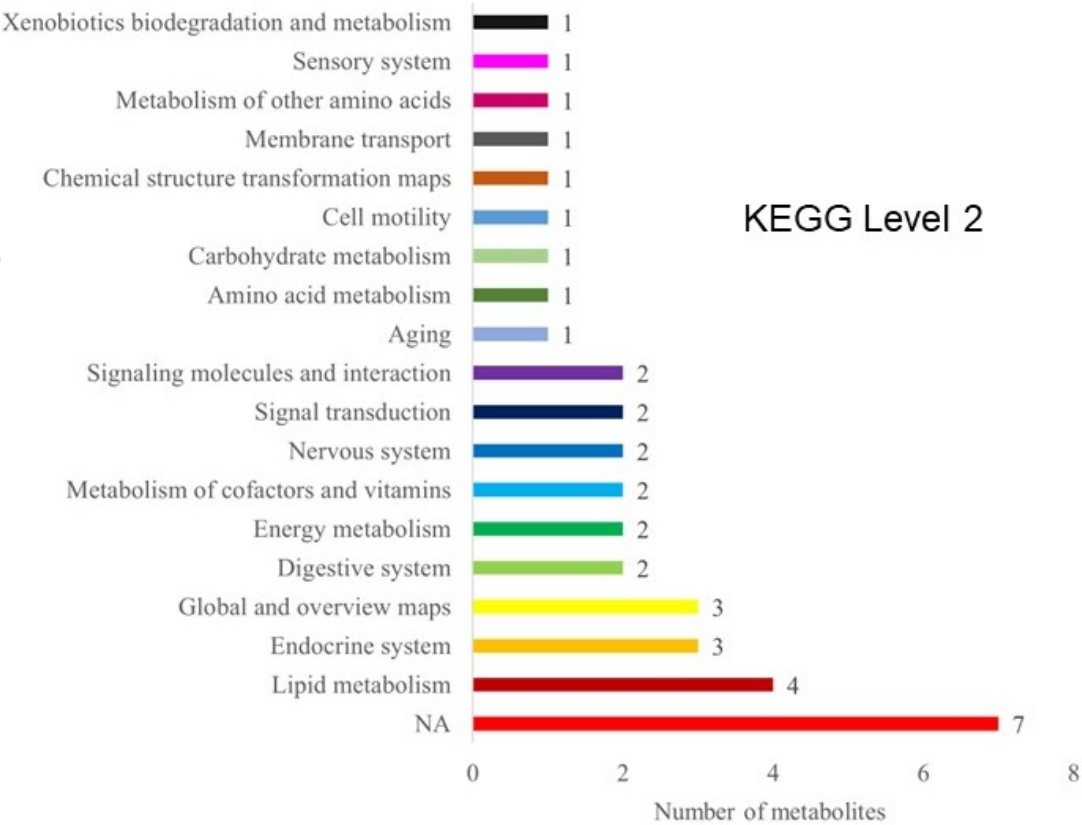
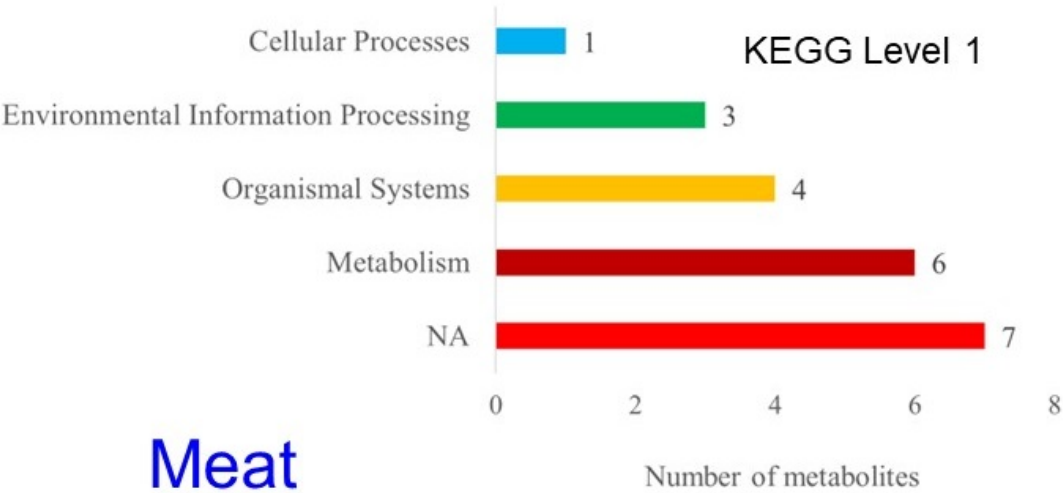


Figure S1. Tissue-specific (meat-specific) metabolic pathways at the KEGG levels 1 and 2 based on the biomarker metabolites shown in Table S5.

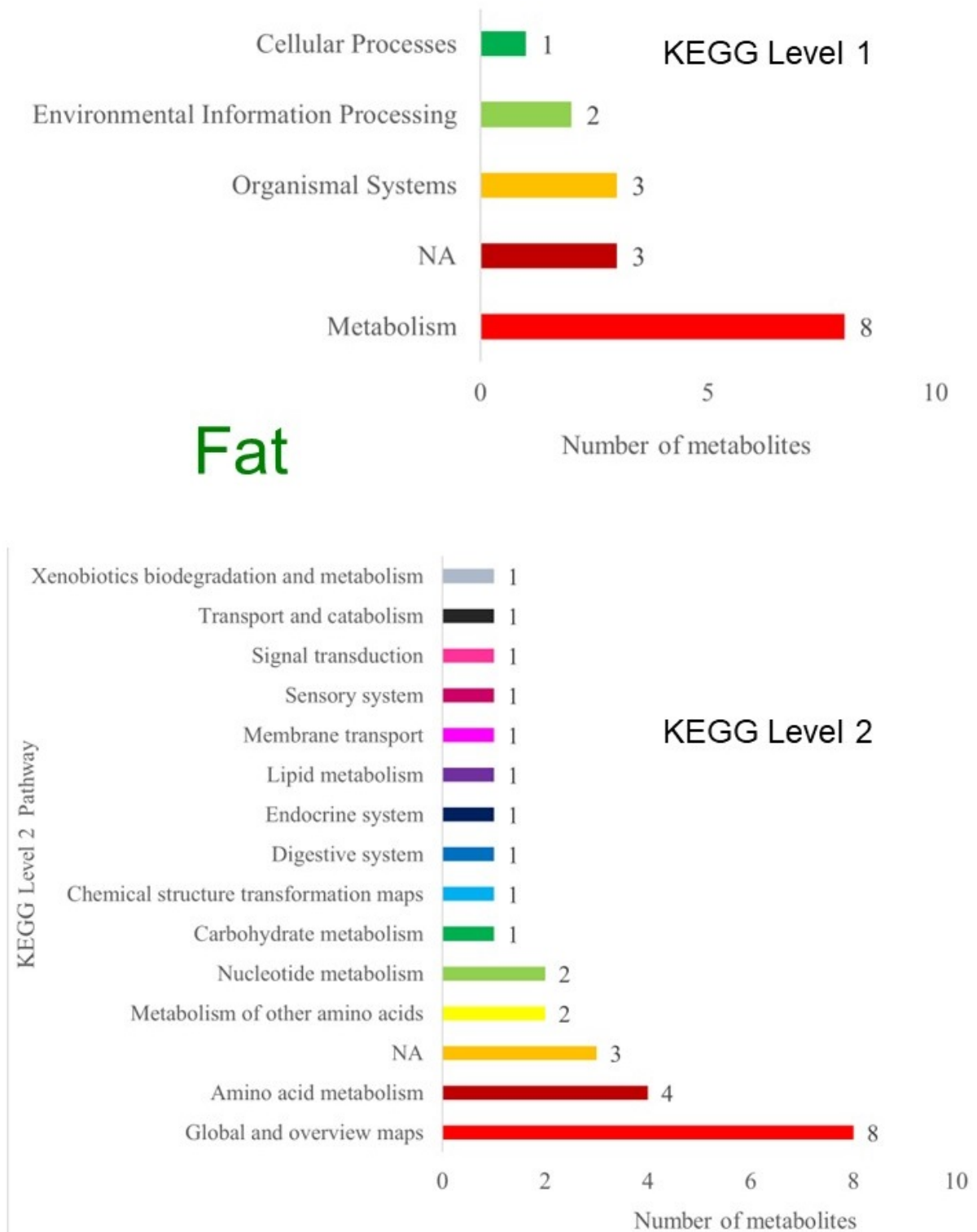


Figure S2. Tissue-specific (fat-specific) metabolic pathways at the KEGG levels 1 and 2 based on the biomarker metabolites shown in Table S5.

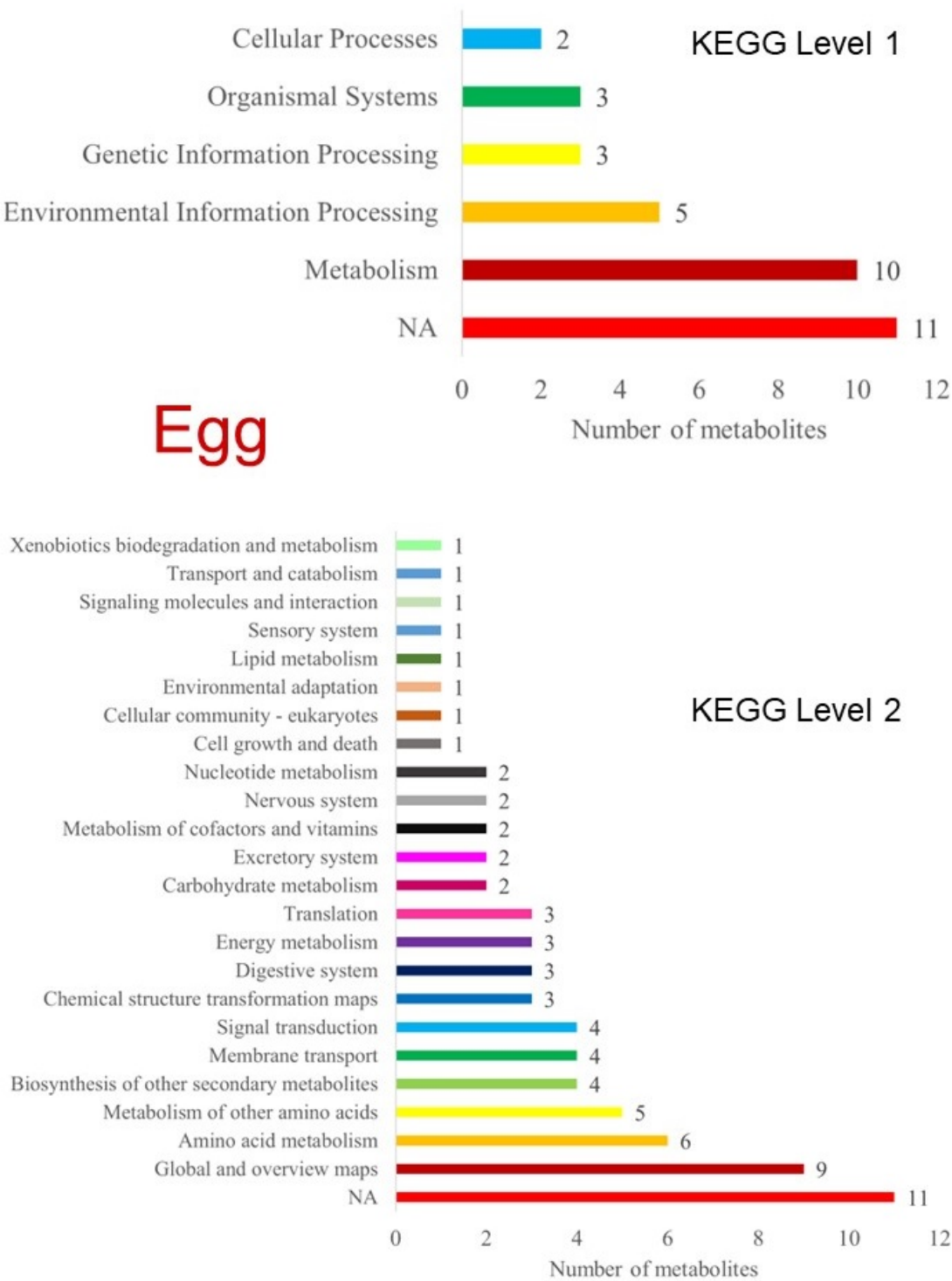


Figure S3. Tissue-specific (fat-specific) metabolic pathways at the KEGG levels 1 and 2 based on the biomarker metabolites shown in Table S5.

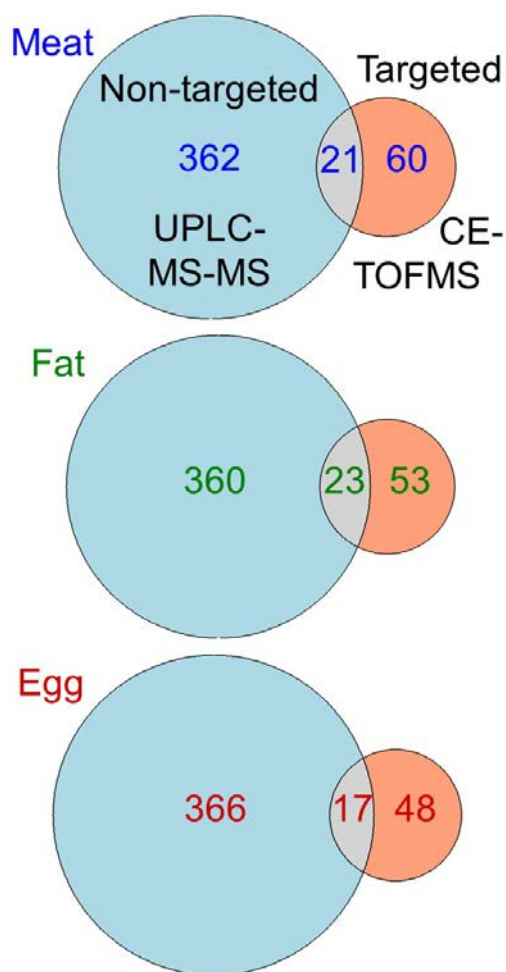


Figure S4. Distribution of the metabolites in meat (topmost), fat (middle), and egg (bottommost) of a Siberian sturgeon, detected by UPLS-MS-MS at BGI and CE-TOFMS at HMT, based on Table S5. All the 383 metabolite species detected by the non-targeted approach were present in meat, fat, and egg tissues. In contrast, only part of the total 245 targeted metabolites were seen in each tissue. Relatively small numbers of metabolites were commonly detected by both approaches. The overall Venn diagram is shown in [Figure 6](#) of the main text.