The benefit-risk analysis of omega-3 polyunsaturated fatty acids and heavy metals in seven smoked fish species from Siberia

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Abstract

In cold smoked species of genus *Coregonus*, identified by molecular genetic analysis, contents of fatty acids and heavy metals and arsenic were measured. The highest content of sum of long-chain polyunsaturated fatty acids of omega-3 family (LC-PUFA), namely eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids, 6.53±0.78 mg g^{-1} wet weight, was characteristic of tugun *Coregonus tugun*. This is the first quantitative estimation (mg LC-PUFA per g of product) of the nutritive value of smoked fish. Thus, to obtain a daily personal doze of

EPA+DHA of 1 g, recommended for prevention of cardiovascular diseases, one needs to consume 153 g of the smoked tugun. Metals contents did not exceed standards for fish meat except Pb in least cisco *Coregonus sardinella*. Accordingly, values of hazard quotients, which estimate benefit-risk ratio of fish intake, indicate that most of the smoked fish species are safe product for human nutrition, except least cisco regarding Pb content.

Keywords: Eicosapentaenoic acid; Docosahexaenoic acid, *Coregonus*, Hazard quotients, Lead, GenBank

1. Introduction

In last decades, long-chain polyunsaturated fatty acids of omega-3 family (LC-PUFA), namely eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) have been recognized as essential compounds for human nutrition as protectors of cardiovascular and neural diseases (Casula et al., 2013; Nagasaka et al., 2014; Dyall, 2015; Weiser et al., 2016). World Health Organization and a number of national health and nutrition organizations recommend for daily personal consumption 0.5 – 1.0 g of EPA+DHA(Harris et al., 2009; Kris-Etherton et al., 2009; Adkins & Kelley, 2010; Nagasaka et al., 2014). The main source of EPA and DHA for humans is fish (Robert, 2006; Adkins & Kelley, 2010; Gladyshev et al., 2013; Gladyshev et al., 2015b). However, content of EPA+DHA in diverse fish species differ more than two orders of magnitude and thereby have severely different nutritive value for humans (Gladyshev et al., 2018). Moreover, culinary treatment may change the nutritive value of fish products (Ruiz-Rodriguez et al., 2008; Zotos et al., 2013; Cheung et al., 2016). Thus, continuous improvement of databases on EPA and DHA content in diverse fish species and products is

necessary in order to reveal the benefits they offer for human health (Usydus & Szlinder-Richert, 2012; Chuang, et al., 2012; Fayet-Moore et al., 2015).

Among freshwater food fish, species of *Coregonus* genus ("whitefish") are known to have one of the highest nutritive values, including the high EPA and DHA contents (Vasconi et al., 2015; Gladyshev et al., 2017, 2018). However, all the above LC-PUFA data were obtained for fresh *Coregonus* fish. In available literature, there is practically no data on EPA and DHA contents (mg g^{-1}) in cooked fish of this genus. It is important to note, that because of tangled phylogenetic features, a visual identification of some species of *Coregonus* genus by morphological traits is very difficult even for skilled ichthyologists (Borovikova et al., 2013), not to mention sellers or purchasers, especially in cases of treated fish products, which lack of many essential morphological details. Consequently, there may be a mistaken labeling of *Coregonus* species during trading. Meanwhile, various species of this genus can differ in LC-PUFA content up to 10-fold (Gladyshev et al., 2017). Thus, to evaluate the nutritive value of *Coregonus* products on shelves, it is necessary to have a reliable method of their identification to verify the label information.DNA barcoding of fish is a well-established method of species identification, including processed fish products (Smith et al., 2008). A number of studies have used a fragment of cytochrome *c* oxidase subunit I (COI) gene for fish products mislabeling assays (Smith et al., 2008; Muñoz-Colmenero et al., 2016; Popa et al., 2017; Pardo et al., 2018). There has never been an attempt of DNA authentication of local fish products on Siberian market.

Besides the benefits of fish consumption, there is also a risk for consumers to get a certain dose of hazardous materials, for instance, heavy metals, which accumulate in fish biomass in polluted environments (Gladyshev et al., 2001; Burger & Gochfeld, 2005; Cheung et al., 2008; Gribble et al., 2016).Thus, it is necessary to quantify benefit – risk ratio of consumption of a portion of a given fish species from a given habitat and then to provide consumers with relevant recommendations (Foran et al., 2005; Budtz-Jorgensen et al., 2007; Gladyshev et al., 2009a).

The aims of present work wereevaluation of the nutritive value of 7 smoked species of *Coregonus* fish, popular in Siberia (Russia) and estimation of benefit-risk ratio of their consumption, basing on contents of LC-PUFA and heavy metals. We hypothesize, that smoking, which was not quantified at present as a factor, affected LC-PUFA contents (mg per g of mass) provides products of the high nutritive value. Besides, an attempt to identify species of the smoked fish products using molecular genetics was made.

2. Materials and methods

2.1. Fish samples

Cold smoked fish were purchased at a local wholesale market at the same time in Krasnoyarsk city (Siberia, Russia). Reputable vendors were chosen to be sure that all the Federal Standards of storage of the products were followed before selling. For following analyses, 10 specimens of fish, labelled as Arctic cisco (*Coregonus autumnalis*) and 5 specimens of each fish, labelled as tugun (*Coregonus tugun*), least cisco (*Coregonus sardinella*), muksun (*Coregonus muksun*), peled (*Coregonus peled*), broad whitefish (*Coregonus nasus*) and common whitefish (*Coregonus lavaretus*) were taken. According to label information, all fish were caught in the Yenisei River basin. Therefore, 40 samples from 40 specimens were analyzed. Muscle tissues without skin (fillets) below the dorsal fin were taken as the samples.

2.2. Analysis of moisture content

To measure moisture content, fillets from the same fish samples of about 10–15 g of wet weight were taken and dried to constant weight at 105 ^{o}C.

2.3. Analysis of fatty acids

Lipids were extracted with chloroform/methanol (2:1, v/v) three times, when tissues were simultaneously homogenized with glass beads (Gladyshev et al., 2014). The extracts were dried with anhydrous Na_2SO_4, and then chloroform and methanol were roto evaporated under vacuum at 35 °C. The extracted lipids were hydrolysed by heating them under reflux at 90°C for 10 min with an excess solution of sodium hydroxide in methanol (8 mg/mL). Then the mixture was cooled for 5 min at room's temperature and added an excess solution of 3% sulphuric acid in methanol and heated under reflux at 90 °C for 10 min to methylate free fatty acids. Finally 5 mL of a saturated solution of NaCl and 3 mL of hexane and were added. Contents were mixed for 1 min, transferred to a separatory funnel, and the lower aquatic layer was discarded. The hexane layer was washed one more time with an aliquot of the solution of NaCl and twice with 5 mL of distilled water. The hexane solution of fatty acid methyl esters (FAMEs) was dried with anhydrous Na_2SO_4, and hexane was removed by roto-evaporating at 35 °C. The FAMEs were redissolved in 150–300 μL of hexane prior to chromatographic analysis.

A gas chromatograph equipped with a mass spectrometer detector (model 7000 QQQ, Agilent Technologies, USA) and with a 30-m long, 0.25-mm internal diameter capillary HP-FFAP column was used for the FAME analysis. . The instrument conditions were following: helium of purity 99.9995% (5.0) as a carrier gas with a constant flow rate of 1 ml min^{-1}; the oven temperature was started at 120 °C for 3 min isothermal, increased to 180°C at a rate of 5° C min^{-1} and hold for 10 min; the next step was to 220°C at a rate of 3°C min^{-1} and hold for 5 min; finally temperature was increased to 230 °C at a rate of 10°C min^{-1} and hold for 30 min. A

volume of 0.5-1 μL of the hexane solution was injected at 250 °C in a split mode with 1:25 split ratio. The mass spectrometer conditions were following: the GC/MS interface, ion source and quadrupole temperatures were 270 °C, 230°C and 180 °C, respectively; single electron impact ionization (EI+) mode at 70 eV was used; scanning of the ion fragments was performed from 45 to 500 atomic units at a rate of 0.5 s $scan^{-1}$. Data were collected in the total ion mode and analyzed using MassHunter Software (Agilent Technologies, USA). Peaks of FAMEs were identified by their mass spectra, comparing them to those in the integrated database NIST 2008 MS LIB (Revision Jan2010) and to those in the standard of 37 FAMEs mixture (U-47885, Supelco, USA). We tested instrumental analytical precision by 5 replicate injections of the standard that gave coefficients of variation of the response values within 0.1-1.1 %.

The FAMEs were quantified according to the peak area of the internal standard, 19:0-FAME (Sigma-Aldrich, USA), which was added to samples in proportion of ~ 1:2000, w/w, prior to the lipid extraction, after addition of the first portion of chloroform/methanol mixture. This approach allowed taking into account all sample processing variability. Using the standard solution U-47885 with addition of solution of the internal standard, 19:0-FAME, we compared response factors for various FAMEs measured with the GC-MS 7000 QQQ with those measured with a gas chromatograph with a flame ionization detector (model 6890, Agilent Technologies, USA) equipped with a column of the same characteristics and worked at similar instrumental conditions. The comparison found close values of response factors for the two FAMEs of interest, 20:5n-3 and 22:6n-3, obtained with the mass spectrometer worked in the total ion mode and the flame ionization detector, and confirmed validity of the quantitative measurements based on the mass spectrometric detection.

2.4. Analysis of heavy metals

Before the analysis, fish samples were dried at 105 ˚C in an oven until constant weight. Element composition of fish was determined using ICP-OES (inductively coupled plasma optic emission spectrometry) analysis with a spectrometer iCAP 6300 Duo (Thermo Scientific, England, 2010). Spectrometer characteristics and analysis procedure were described in details in (Anishchenko et al., 2017). Briefly, 0.2 g of dried fish samples were digested in 5 mL of HNO_3:$HClO_4$ (1:1, analytical grade) on a laboratory hotplate. ICP multi-element solutions IV and XVI (Merck, Darmstadt, Germany) were diluted for HM calibration standards. Pure chemicals for spectrometry (CaO, MgO, KCl, Na_2SO_4) were used for the HM calibration as matrix elements, corresponded to the samples. ICP multi-elements solutions (Merck, Darmstadt, Germany) and pure chemicals for spectrometry (CaO, MgO, KCl, Na_2SO_4, $(NH_4)_2HPO_4$) were used for calibration standards. Scandium (5 mg/L, Scandium Standard for ICP, Fluka, Switzerland) was used as an internal standard. Samples and standards were diluted with deionized water (18 MΩ). All samples were measured in duplicate. Quality of the sample preparation and element detection was controlled using Standard Reference Material (SRM) No 9055-2008 «Muscles of Baikal perch» (Vinogradov Institute of Geochemistry SB RAS, Irkutsk, Russia). Recovery rate of As in the SRM was 90%, Cd – 89%, Cu – 81%, Fe – 127%, Mn – 78%, Zn – 129% and 110% for Pb, 83% for Cr, 72% for Co, 62% for Ni (values of Pb, Cr, Co and Ni were noted as approximate in the SRM certificate). LOD/LOQ for each heavy metal in the ICP-OES analysis are presented in Table 1.

2.5. Molecular genetic analysis

DNA was extracted following a standard phenol-chloroform protocol as in (Smith et al., 2008). A fragment of cytochrome c oxidase subunit gene was amplified using primers FishF1 (5’CCAACCACAAAGACATTGGCAC) and FishR1 (5’-

ACTTCTGGGTGGCCAAAGAATCA). Amplifications were carried out using an initial denaturation of 94° C for 3 min; 30 cycles of 94° C for 30 s, 60° C for 30 s and 72° C for 90 s, followed by an extension at 72° C for 5 min, using a Dyad DNA Engine thermal cycler (BioRad, CA, USA). Polymerase chain reaction (PCR) mixture consisted of 0.8 U recombinant Taq DNA polymerase in a corresponding 1× reaction buffer (Thermo Fischer Scientific, MA, USA), 1.5 μM Mg^{2+}, 0.25 mM of each dNTPs, 0.25 μM of each primer, and 2 μL of extracted DNA in 25 μL of total volume. Ethidium bromide stained PCR products were visualized under ultraviolet light in 1.2% agarose gels after electrophoresis at 100 V for 30 min. The amount of DNA was quantified by AccuLite 470 Mini Fluorometer (Biotium, CA, USA).

PCR products were purified of unincorporated primers and dNTPs with Illustra ExoStar PCR and Sequence Reaction Clean-Up Kit (GE Healthcare, IL, USA). PCR products were sequenced following Thermo Sequenase Labeled Primers Cycle Sequencing protocol and run on a 4300L DNA Analyzer (Li-Cor, NE, USA).

DNA sequences were edited and aligned in BioEdit sequence alignment editor (Hall, 1999), and then queried using online BLAST (Basic Local Alignment Search Tool) against GenBank [https://www.ncbi.nlm.nih.gov/genbank/] COI entries for the fish species which were indicated on the specimen labels. Additionally, we used the Identification engine on BOLD (Barcode of Life Data Systems) (Ratnasingham & Hebert, 2007) to obtain a list of records with the highest similarity to the analyzed sequences.The sequence is considered identified when a ≥98% match with a known species is found in available online databases (Popa et al., 2017).

2.5. Calculations of hazard quotient for benefit-risk ratio

The benefit-risk ratio was expressed as the dimensionless hazard quotient, HQ_{EFA} (Gladyshev et al., 2009a):

$$HQ_{EFA} = \frac{R_{EFA} \cdot c}{C \cdot RfD \cdot AW} \quad (1)$$

where R_{EFA} (mg · day^{-1}) is the recommended daily dose of essential fatty acids (EFA) for a human person, c (μg · g^{-1}) is content of a given metal and arsenic in a given fish product, C (mg · g^{-1}) is content of EFA (EPA+DHA) in a given fish product, RfD (μg · kg^{-1} ·day^{-1}) is a reference dose, i.e., a dose of a specific metal known to result in no deleterious health effects and AW (kg) is an average adult weight. $HQ_{EFA} < 1$ means the health benefit from fish consumption, and $HQ_{EFA} > 1$ means the risk (Gladyshev et al., 2009a). This equation was successfully used in a number of studies (Zhang et al., 2012; Razavi et al., 2014; Strandberg et al., 2016). For the calculations by Equation 1, we used R_{EFA} = 1000 mg ·day^{-1}, AW = 70 kg and median RfD values, given in Anishchenko et al. (2017).

2.6. Statistical analysis

One-way ANOVA with Tukey HSD *post hoc* test, Kruskal-Wallis test and multivariate correspondence analysis (Legendre & Legendre, 1998) were calculated conventionally, using STATISTICA software, version 9.0 (StatSoft, Inc., Tulsa, OK, USA). Only normally distributed variables (Kolmogorov-Smirnov one-sample test for normality) were included in ANOVA, while other variables were compared using the non-parametric Kruskal-Wallis test.

3. Results

231 *3.1. Genetic phenotyping*

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233 Using the barcoding, most of the analysed samples were only identified down to genus
234 level. However, no possible mismatches were discovered. For all samples, the species designated
235 on the label were among the most highly similar BLAST search results. The retrieved sequences
236 were deposited in GenBank under the following accession numbers: MH823497-MH823538.
237 *C. tugun* was the only species unambiguously attributed by both GenBank and BOLD to
238 the fish species indicated on the label. For this species, there is only one barcode sequence
239 available in the databases. For samples labeled as broad whitefish Genbank gave 100% identity
240 to *C. nasus,* whereas BOLD was unable to unambiguously classify these samples, even though
241 *C. nasus* was also the only suggested match with >99% identity.
242 COI sequences of Arctic cisco samples were 100% identical to *C. pollan* and C.
243 *autumnalis* on Genbank. *C. pollan* is endemic to several Irish lakes and can be excluded from the
244 suspects. Again, BOLD suggested a number of *Coregonus* species as possible identities.
245 Common whitefish, muksun, peled and least cisco were not identified to species level
246 neither by BOLD nor Genbank, because they share 99-100% identity with numerous *Coregonus*
247 species.

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249 *3.2. Fatty acids*

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251 The canonical correspondence analysis of fatty acid (FA) percentages in the tissues
252 demonstrated a considerable partitioning of some fish species (Fig. 1). Along Dimension 1,
253 which represented the largest proportion of inertia, 38.4%, most overall differences in FA
254 composition were found between *C. autumnalis* and *C. nasus* (Fig. 1). These differences were
255 mainly provided by contrast levels of sum 22:1 and 18:3n-3 in the species (Fig. 1). Along
256 Dimension 2 (21.3% of inertia), most differences were between *C. autumnalis* and *C. peled* (Fig.

1). These differences were primarily due to the contrast between levels of sum 22:1 and 22:5n-6 in the species (Fig. 1). Along both dimensions, *C. autumnalis* had comparatively high variation (Fig. 1), which indicated considerable differences in FA composition of the purchased smoked specimens. Indeed, among 10 specimens of *C. autumnalis*, one had zero level of the indicator FA, sum 22:1, as well as extremely low content of EPA+DHA, 0.46 $mg \cdot g^{-1}$.

C. sardinella tended to have the highest mean levels of 14:0 and 16PUFA (sum of polyunsaturated fatty acids with 16 carbon atoms), but the lowest levels of 20:2n-6 and 20:3n-3 (Table 2). *C. peled* tended to have the highest levels of 16:1n-9 and 18:4n-3, but the lowest level of 16:1n-7 (Table 2). *C. autumnalis* tended to have the highest level, of 16:1n-7, sum 20:1 and sum 22:1, but the lowest levels of 18:2n-6 and 22:5n-6 (Table 2). *C. tugun* tended to have the highest levels of 18:0 and 18:1n-9, but the lowest levels of 20:5n-3 and 22:5n-3 (Table 2). *C. muksun* tended to have the highest levels of 18:1n-7, 20:4n-3 and 22:4n-3 (Table 2). *C. nasus* tended to have the highest levels of 18:2n-6 and 20:2n-6 (Table 2). *C. lavaretus* tended to have the highest level of 22:5n-6 (Table 2). Mean levels of 18:3n-3 in *C. nasus*, *C. tugun* and *C. peled* were significantly higher than those in *C. autumnalis*, *C. muksun* and *C. sardinella* (Table 2). Mean levels of 20:4n-6 (marker of terrestrial inputs) in *C. nasus*, *C. peled* and *C. lavaretus* were significantly higher than those in *C. autumnalis*, *C. muksun*, *C. tugun* and *C. sardinella* (Table 2). *C. tugun* tended to have the highest total contents ($mg \cdot g^{-1}$ of wet weight) of fatty acids (Table 2).

Mean contents of EPA+DHA in the studied species varied from 1.83 ± 0.32 mg g^{-1} wet weight in whitefish *C. lavaretus* to 6.53 ± 0.78 mg g^{-1} in tugun *C. tugun* (Fig. 2). Thus, to obtain the recommended daily intake of EPA+DHA, 1 $g \cdot day^{-1}$, one needs to consume portions of smoked fish from ~150 g of tugun to ~550 g of whitefish (Table 3).

Moisture content of studied species had a modest range of variations. *C. tugun* had the lowest mean value of moisture, $51.7 \pm 1.8\%$, while whitefish *C. lavaretus* had the highest value, $71.4 \pm 1.3\%$.

283

284 *3.3. Heavy metals*

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286 *C. autumnalis* tended to have the highest mean content of As (Table 4). The highest
287 content of Co tended to be characteristic of *C. nasus* (Table 4). *C. sardinella* tended to have the
288 highest contents of Cr and Pb, while *C. tugun* tended to have the highest contents of Cu and Se
289 (Table 4). *C. muksun* tended to have the lowest content of Fe, while the lowest content of Zn
290 tended to be characteristic of *C. lavaretus* (Table 4). *C. peled* tended to have the lowest content
291 of Mn, while *C. sardinella* tended to have the highest content of this metal (Table 4).

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293 *3.4. Benefit-risk ratio*

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295 Values of hazard quotients, HQ_{EFA}, are given in Table 5. In most species, $HQ_{EFA} < 1$,
296 therefore there is no risk for people to eat these fish (Table 5). However, in least cisco *C.*
297 *sardinella*, HQ_{EFA} for Pb > 1, which means that risk of consumption of this fish over weighted
298 benefits of EPA+DHA intakes (Table 5).

299

300

301 **4. Discussion**

302

303 The benefit-risk analysis revealed that most smoked fish species of genus *Coregonus*,
304 commonly often designated as “whitefish”, caught in the lower Yenisei River, are very valuable
305 healthy products. On the one hand, their valuable properties were caused by the high content of
306 LC-PUFA, on the other hand – by the low contents of heavy metals. LC-PUFA contents in the
307 smoked whitefish, 1.8 – 6.5 mg g^{-1}, were higher, than those in many other fish products, popular
308 in diverse regions (Table 6). It is worth to note that nutritive value of products for humans have

309 to be estimated on the basis of contents of LC-PUFA, i.e., mass units (mg per g of product),
310 rather than relative levels (% of total FA) (Gladyshev et al., 2007, Gladyshev et al., 2015b;
311 Huynh & Kitts, 2009; Woods & Fearon, 2009; Joordens et al., 2014). However, the quantitative
312 estimations are scarce (Table 6) and most data are published as the percentages leading to
313 erroneous conclusions on nutritive values of studied products for humans (Gladyshev et al.,
314 2007, 2015b; Woods et al., 2009; Turchini et al., 2018). Our present data seems to be the first
315 quantification of the nutritive value of smoked fish in mass units.

316 Fatty acid composition and content of most Coregonus species as raw material from this
317 region were previously studied (Gladyshev et al., 2017). Although the raw fish from our previous
318 study (Gladyshev et al., 2017) and the present smoked specimens cannot be compared directly, it
319 is worth to note that total fatty acid profiles of the smoked fish resembled those of the relevant
320 raw species. Indeed, mean levels 18:3n-3, which is the biomarker of trophic chains based on
321 green algae and cyanobacteria (Gladyshev et al., 2015a) in smoked *C. nasus*, *C. tugun* and *C.*
322 *peled* were significantly higher than those in *C. autumnalis* and *C. sardinella*, and exactly the
323 same pattern was characteristic of previously studied raw fish of these species from the Yenisei
324 river (Gladyshev et al., 2017). Moreover, mean levels 20:4n-6, which is the biomarker of trophic
325 chains, based on inputs of terrestrial organic matter (Gladyshev et al., 2015a), in smoked *C.*
326 *nasus*, *C. peled* and *C. lavaretus* were significantly higher than those in *C. autumnalis*, *C. tugun*
327 and *C. sardinella*, just like that in previously studied raw fish of these species from the Yenisei
328 river (Gladyshev et al., 2017). Besides, smoked *C. autumnalis* tended to have the highest level of
329 sum 20:1 and sum 22:1, which are the biomarkers of marine copepods, since this species
330 migrates to the Yenisei Estuary for feeding (Gladyshev et al., 2017). Thus, basing on the
331 biomarker fatty acids, it is reasonable to conclude that smoked species studied in the present
332 work, were really caught in the Yenisei River, as noted in their sale labels. Moreover, their
333 species identities were confirmed by the molecular genetic analysis.

Although fatty acid profiles of the presently studied smoked fish of the *Coregonus* genus had considerable resemblance with those of the raw specimen, contents of EPA and DHA in most smoked fish species appeared to be lower than those of raw fish from our previous study (Gladyshev et al., 2017). Could these low contents be a result of the culinary treatment? There are many evidences that certain culinary treatments do not cause a decrease of LC-PUFA contents (mg g^{-1}) in some fish (Candella et al., 1998; Gladyshev et al., 2006; Gladyshev et al., 2007, 2014; Ansorena et al., 2010; Leung et al., 2018, but see Candella et al., 1998; Sioen et al., 2006; Gladyshev et al., 2007; Cheung et al., 2016). However, among the numerous ways of culinary treatments (boiling frying, etc.), tested in the cited above studies, there was no smoking. To our knowledge, an effect of smoking on LC-PUFA content (mg g^{-1}) in fish was not reported yet in available literature. Thus, it will be worth to find out in future studies, if smoking really decreases EPA and DHA contents in fish and thereby another ways of culinary treatment of *Coregonus* fish (e.g., boiling, stewing) should be recommended.

The studied smoked fish had considerably good nutritive value, regarding contents of EPA+DHA compared to many other fish products (Table 6). According to a classification of nutritive value based on LC-PUFA content (Fayet-Moore et al., 2015), most studied fish species, *C. tugun*, *C. autumnalis*, *C. sardinella*, *C. muksun*, *C. peled* and *C. nasus* can be characterized as "good", while *C. lavaretus* appeared to be "moderate". To obtain the recommended daily personal intake of EPA+DHA of 1 g (Harris et al., 2009; Kris-Etherton et al., 2009; Adkins & Kelley, 2010; Nagasaka et al., 2014), one needs to consume portions of smoked whitefish of *Coregonus* genus ca. 200 g.

The nutritive value of some studied smoked fish may be diminished due to excessive content of metals and arsenic. Total (sum of organic and inorganic forms) As content in *C. autumnalis* and *C. muksun* exceeded Russian threshold limit value (TLV) for freshwater fish (Hygienic…, 2001), Argentinean legislation limit for food (1 mg kg^{-1}, Schenone et al., 2013) and Serbian limit for freshwater and marine fish (2 mg kg^{-1}, Novakov et al., 2017) for total arsenic.

European Union and World Health Organization (WHO) have no recommendation for limit content of total and inorganic As in fish and fish products. Inorganic arsenic form is more toxic then organic, but its content in fish, as a rule, is only 1-5 % of total As (Schenone et al., 2013). Maximum inorganic arsenic intake was estimated as 4 % of total As, in a case of high fish consumption (US EPA, 1997). Arsenobetain and arsenocholine are major As-contained organic compounds in freshwater fish, and have no tendency for bioconcentration (US EPA, 1997). Arsenic can enter to a fish organism with water and food. High content of As in fish muscles were reported for specimens from environment contaminated due to mining activity (Culioli et al., 2009). Mining takes place in the lower Yenisei River watershed and might be a cause of increased As content in some studied fish.

Pb content in smoked *C. sardinella* was five times higher than European, Serbian (Novakov et al., 2017) and Food Agricultural Organization (FAO)/WHO limits (0.3 mg kg^{-1}) (Codex Alimentarius, 2015), and exceeded Canadian standards (0.5 mg kg^{-1}) (Hursky & Pietrock, 2012) and Russian TLV (1 mg kg^{-1}) (Hygienic…, 2001). Cd in *C. sardinella* was also 2.6 times higher than European and Serbian recommended upper limit (0.05 mg kg^{-1}) (Novakov et al., 2017), but did not exceed Russian TLV (0.2 mg kg^{-1}) (Hygienic…, 2001).

Heavy metals and arsenic contents of smoked *C. lavaretus* did not exceed those (when comparing per dry weight units) in raw whitefish from lentic and lotic ecosystems in areas where nickel plants are located: Ni, Cu, Cd, Zn, Pb in *C. lavaretus* of Kola peninsula (Amundsen et al., 2011) and As, Ni, Cd, Zn, Pb, Se in *Coregonus* spp. of Taimyr Peninsula (Allen-Gil et al., 2003). Cu content in the studied smoked fish was higher than that in whitefish of Taimyr Peninsula. Cu and Zn in the smoked fish did not exceed permissible limits according to FAO (30 mg kg^{-1}) (Varol & Sünbül, 2019). Pb content in smoked *C. sardinella* slightly exceeded average that in *Coregonus sardinella valenciennes* fresh fillet from the Indigirka River (Republic Sakha, Russia) (Abramov et al., 2015, recalculated for given moisture 74.6%). Cd content in smoked *C. sardinella* was equal to that in the fish from the Indigirka River (Abramov et al., 2015). Contents

386 of essential metals (Fe, Mn, Cu, Zn, Se) in smoked *C. sardinella* and *C. tugun* and content of Zn
387 in smoked *C. muksun*, *C. nasus* and *C. peled* were higher than average values for many raw fish
388 specimen (Tacon & Metian, 2013).

389 Increase of element contents in the smoked fish might be due to a contamination during
390 fuel combustion in the smoking process (Codex Alimentarius, 2009). For instance, Pb content in
391 common carp, rainbow trout and northern pike were found to increase after smoking in
392 comparison with the raw fish specimens (Cieślik et al., 2018). Similarly, the high Pb content in
393 least cisco found in our work might be the result of smoking.

394 The high content of a number of metals in some fish species mentioned above did not
395 diminish their nutritive value, if take into account the benefit-risk ratio, namely the obtained
396 values of hazard quotients HQ_{EFA}<1. Indeed, except Pb in least cisco, all the portions of the
397 smoked fish, which provide a consumer with the recommended daily dose of EPA+DHA of 1 g,
398 contained the harmless quantity of heavy metals. Thus, it is worth to note that the quantification
399 of benefit-risk ratio of the products seems to be more precise way of estimation of their nutritive
400 value, than using threshold limit values alone. Taking into account the hazardous content of Pb
401 in least cisco, a monitoring of smoked whitefish on the basis of the hazard quotients can be
402 recommended to provide consumers with the necessary information for healthy product choice.
403
404
405 **5. Conclusions**
406
407 Contents of EPA and DHA, mg per g of product, were quantified in smoked fish for the
408 first time. The studied smoked fish species of *Coregonus* genus from the Yenisei River, which
409 are popular common food fish in Krasnoyarsk Region (Siberia, Russia), appeared to be of high
410 nutritive value concerning EPA and DHA content. For consumers, the benefit from PUFA
411 content in the smoked fish overweighed the risk due to contents of heavy metals and arsenic,

except one case. For future studies, it is worth to compare the smoking with another popular ways of fish culinary treatments concerning their effects on EPA and DHA contents and the benefit-risk ratio.

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Declaration of Competing Interest

Authors do not have any conflicts of interest to disclose.

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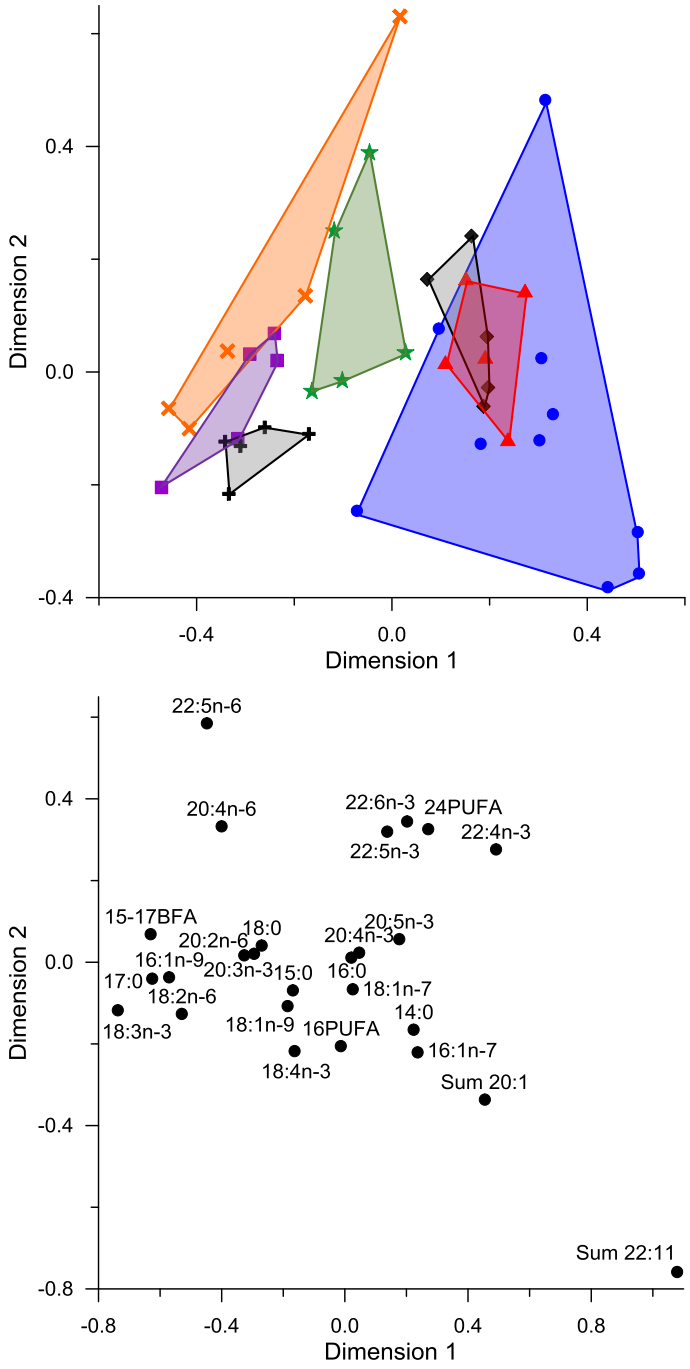
Figure legends

Fig.1. Canonical correspondence analyses of fatty-acid (FA) composition (% of the total) in smoked fish: circles – Arctic cisco *Coregonus autumnalis*, triangles – muksun *C. muksun*, squares – broad whitefish *C. nasus*, crosses – tugun *C. tugun*, oblique crosses – peled *C. peled*, diamonds – least cisco *C. sardinella*, stars – whitefish *C. lavaretus*.

Fig 2. Mean content ($mg \cdot g^{-1}$ wet weight) of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and their sum (EPA+DHA) in smoked fish species: aut – Arctic cisco *Coregonus autumnalis*, muk – muksun *C. muksun*, nas – broad whitefish *C. nasus*, tug – tugun *C. tugun*, pel – peled *C. peled*, sar – least cisco *C. sardinella* and lav – whitefish *C. lavaretus*. Bars represent standard error. Means labelled with the same letter are not significantly different at $P < 0.05$ after ANOVA and Tukey HSD *post hoc* test.

Fig.1.



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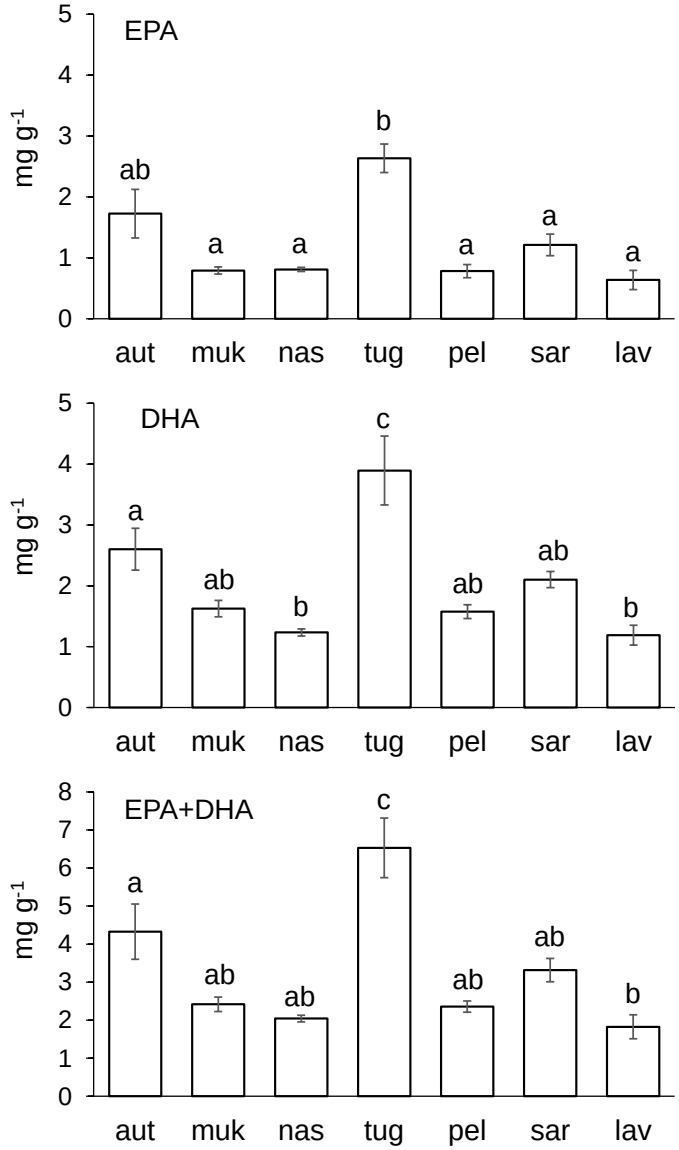
642 **Fig 2.**



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646 **Table 1.**

647 Limit of detection (LOD, mg · L^{-1}) and limit of quantitation (LOQ, mg · L^{-1}) of the ICP-OES method.

Element	λ, nm	LOD	LOQ
As	189.402	0.001	0.004
Cd	214.438	0.0001	0.0004
Co	228.616	0.0003	0.0009
Cr	267.716	0.0002	0.0007
Cu	324.754	0.0001	0.0005
Fe	238.204	0.0002	0.0005
Mn	257.610	0.00004	0.00012
Ni	231.604	0.0007	0.0022
Pb	220.353	0.002	0.007
Se	196.090	0.002	0.006

Sn	189.989	0.0007	0.0025
Zn	213.856	0.0001	0.0004

Table 2

Mean values of percentages (% of total fatty acids ± standard error) and total contents (Total, mg g^{-1} of wet weight) of fatty acids in studied smoked fish species: Arctic cisco *Coregonus autumnalis* (number of samples, n = 10), muksun *C. muksun* (n = 5), broad whitefish *C. nasus* (n = 5), tugun *C. tugun* (n = 5), peled *C. peled* (n = 5), least cisco *C. sardinella* (n = 5) and whitefish *C. lavaretus* (n = 5). Normally distributed variables are compared by ANOVA and Tukey HSD *post hoc* test; the other variables marked with* and are compared by Kruskal-Wallis test. Means labelled with the same letter are not significantly different at $P<0.05$ according to the relevant test. When ANOVA or Kruskal-Wallis test are insignificant, letter labels are absent.

Fatty acid	*C. autumnalis*	*C. muksun*	*C. nasus*	*C. tugun*	*C. peled*
14:0	3.4 ± 0.3^{A}	3.8 ± 0.4^{AB}	1.9 ± 0.2^{A}	2.6 ± 0.2^{A}	2.2 ± 0.4^{A}
15:0	0.3 ± 0.0^{AB}	0.4 ± 0.0^{AB}	0.4 ± 0.1^{AB}	0.5 ± 0.1^{B}	0.4 ± 0.1^{AB}
16:0	15.4 ± 0.9	15.9 ± 1.0	13.4 ± 0.6	15.1 ± 0.5	13.3 ± 0.8
16:1n-9	0.2 ± 0.0^{A}	0.2 ± 0.0^{A}	0.9 ± 0.1^{BC}	0.6 ± 0.1^{CD}	1.0 ± 0.1^{B}
16:1n-7	14.7 ± 1.4^{A}	13.3 ± 1.9^{AB}	9.3 ± 0.6^{BC}	8.8 ± 0.3^{BC}	7.0 ± 0.6^{C}
15-17BFA[1]	0.5 ± 0.1^{A}	0.6 ± 0.1^{A}	2.5 ± 0.2^{B}	1.2 ± 0.1^{A}	2.8 ± 0.7^{B}
16PUFA	1.2 ± 0.1^{A}	0.7 ± 0.1^{A}	1.3 ± 0.1^{AB}	1.0 ± 0.1^{A}	1.3 ± 0.3^{AB}
17:0	0.1 ± 0.0^{A}	0.1 ± 0.0^{A}	0.6 ± 0.0^{B}	0.6 ± 0.1^{B}	0.6 ± 0.1^{B}
18:0	3.0 ± 0.4^{A}	2.6 ± 0.2^{A}	4.2 ± 0.1^{AC}	6.3 ± 0.3^{B}	4.7 ± 0.2^{BC}
18:1n-9	14.1 ± 1.5^{A}	13.2 ± 0.6^{A}	16.6 ± 1.2^{A}	23.2 ± 0.5^{B}	17.3 ± 1.9^{AB}
18:1n-7	5.4 ± 0.5^{AB}	6.8 ± 0.6^{A}	5.2 ± 0.2^{AB}	5.8 ± 0.2^{AB}	4.1 ± 0.2^{B}
18:2n-6*	1.3 ± 0.1^{A}	1.6 ± 0.2^{AB}	5.6 ± 0.5^{B}	4.9 ± 0.4^{BC}	3.1 ± 0.4^{AB}
18:3n-3	0.8 ± 0.2^{A}	1.4 ± 0.4^{A}	5.5 ± 0.9^{B}	4.1 ± 0.2^{BC}	5.2 ± 1.0^{B}
18:4n-3	1.4 ± 0.2^{AB}	1.4 ± 0.3^{AB}	1.1 ± 0.1^{A}	1.3 ± 0.1^{AB}	2.5 ± 0.6^{B}
Sum 20:1*	4.9 ± 0.8^{A}	2.6 ± 0.4^{AB}	2.2 ± 0.2^{AB}	1.7 ± 0.1^{AB}	0.8 ± 0.0^{B}
20:2n-6	0.3 ± 0.0^{AC}	0.5 ± 0.1^{AB}	0.7 ± 0.1^{B}	0.5 ± 0.0^{AB}	0.2 ± 0.0^{AC}
20:4n-6	0.8 ± 0.1^{A}	0.8 ± 0.1^{A}	3.5 ± 0.4^{B}	1.7 ± 0.2^{A}	3.1 ± 0.5^{B}
20:3n-3	0.2 ± 0.0^{AB}	0.5 ± 0.2^{A}	0.5 ± 0.1^{A}	0.4 ± 0.0^{AB}	0.4 ± 0.1^{AB}
20:4n-3	0.9 ± 0.1^{AB}	1.3 ± 0.3^{A}	0.6 ± 0.1^{B}	0.6 ± 0.1^{AB}	0.9 ± 0.2^{AB}
20:5n-3	8.5 ± 0.6^{A}	8.0 ± 0.8^{AB}	6.6 ± 0.7^{AB}	5.5 ± 0.3^{B}	6.7 ± 0.6^{AB}
Sum 22:1*	1.6 ± 0.5^{A}	0.2 ± 0.1^{AB}	0.2 ± 0.0^{AB}	0.1 ± 0.0^{AB}	0.1 ± 0.0^{B}
22:5n-6*	0.1 ± 0.0^{A}	0.2 ± 0.0^{AB}	0.6 ± 0.1^{ABC}	0.4 ± 0.1^{ABC}	1.1 ± 0.2^{BC}
22:4n-3*	0.2 ± 0.1^{AB}	0.7 ± 0.3^{A}	0.0 ± 0.0^{B}	0.1 ± 0.0^{AB}	0.1 ± 0.0^{AB}
22:5n-3	2.1 ± 0.2^{AB}	2.3 ± 0.3^{AB}	1.7 ± 0.2^{AB}	1.2 ± 0.1^{A}	2.0 ± 0.5^{AB}
22:6n-3	15.2 ± 2.2	16.3 ± 1.2	10.2 ± 1.3	8.0 ± 0.9	15.6 ± 4.2
24PUFA*	0.9 ± 0.2	1.6 ± 0.5	0.3 ± 0.0	0.5 ± 0.1	0.5 ± 0.1
Total*, mg·g^{-1}	19.0 ± 3.5^{AB}	10.2 ± 1.1^{A}	13.0 ± 1.9^{AB}	48.0 ± 2.1^{B}	12.3 ± 2.1^{AB}

[1] 15-17BFA, sum of branched fatty acids with 15 and 17-carbon atom chains.

Table 3

The quantity of smoked fish to be consumed for obtaining the recommended appropriate intake of sum of eicosapentaenoic and docosahexaenoic fatty acids for humans, 1 g day^{-1}.

Species	Quantity, g
Arctic cisco *Coregonus autumnalis*	231

Muksun *Coregonus muksun*	413
Broad whitefish *Coregonus nasus*	489
Tugun *Coregonus tugun*	153
Peled *Coregonus peled*	424
Least cisco *Coregonus sardinella*	302
Witefish *Coregonus lavaretus*	548

Table 4

Mean contents (mg kg^{-1} wet weight ± standard error) of metals and arsenic in studied smoked fish species: Arctic cisco *Coregonus autumnalis*, muksun *C. muksun*, broad whitefish *C. nasus*, tugun *C. tugun*, peled *C. peled*, least cisco *C. sardinella* and whitefish *C. lavaretus*. Normally distributed variables are compared by ANOVA and Tukey HSD *post hoc* test; the other variables marked with* and are compared by Kruskal-Wallis test. Means labelled with the same letter are not significantly different at $P<0.05$ according to the relevant test. When ANOVA or Kruskal-Wallis test are insignificant, letter labels are absent.

Metal	*C. autumnalis*	*C. muksun*	*C. nasus*	*C. tugun*	*C. peled*
As*	3.29 ± 0.39^{A}	2.15 ± 0.23^{B}	0.06 ± 0.01^{BC}	0.07 ± 0.04^{ABC}	0.06 ± 0.01^{BC}
Cd*	0.01 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}
Co*	0.01 ± 0.00^{AB}	0.02 ± 0.00^{AB}	0.03 ± 0.01^{A}	0.00 ± 0.00^{AB}	0.01 ± 0.00^{AB}
Cr	0.14 ± 0.01^{AB}	0.10 ± 0.02^{A}	0.09 ± 0.02^{A}	0.11 ± 0.01^{AB}	0.06 ± 0.01^{A}
Cu	0.45 ± 0.03^{AC}	0.34 ± 0.02^{A}	0.42 ± 0.05^{AC}	0.97 ± 0.02^{BC}	0.44 ± 0.06^{AC}
Fe*	4.58 ± 0.56^{AB}	1.49 ± 0.39^{A}	4.89 ± 0.49^{AB}	12.41 ± 0.62^{B}	4.70 ± 0.53^{AB}
Mn*	0.18 ± 0.01^{AB}	0.20 ± 0.03^{AB}	0.14 ± 0.03^{AB}	0.21 ± 0.01^{AB}	0.10 ± 0.02^{A}
Ni	0.05 ± 0.02	0.06 ± 0.02	0.11 ± 0.04	0.00 ± 0.00	0.02 ± 0.01
Pb*	0.29 ± 0.12^{AB}	0.02 ± 0.01^{AB}	0.08 ± 0.02^{AB}	0.00 ± 0.00^{AB}	0.00 ± 0.00^{AB}
Se	0.15 ± 0.03^{A}	0.29 ± 0.04^{A}	0.22 ± 0.08^{A}	0.64 ± 0.07^{B}	0.24 ± 0.01^{A}
Sn*	0.05 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.04 ± 0.01
Zn*	5.22 ± 0.33^{AC}	9.02 ± 2.06^{ABC}	7.99 ± 0.65^{ABC}	25.85 ± 2.18^{AB}	7.31 ± 0.61^{ABC}

Table 5

Hazard quotients, HQ_{EFA}, for benefit-risk ratio of essential fatty acids vs. heavy metals and arsenic for intake of smoked fish: Arctic cisco *Coregonus autumnalis*, muksun *C. muksun*, broad whitefish *C. nasus*, tugun *C. tugun*, peled *C. peled*, least cisco *C. sardinella* and common whitefish *C. lavaretus*.

Вид	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Se	Sn	Zn
Arctic cisco	0.48	0.05	0.00	0.27	0.07	0.05	0.01	0.01	0.28	0.14	0.00	0.12
Muksun	0.39	0.00	0.02	0.22	0.05	0.01	0.01	0.02	0.03	0.36	0.00	0.18
Broad whitefish	0.01	0.01	0.04	0.22	0.07	0.05	0.01	0.04	0.16	0.30	0.00	0.19
Tugun	0.01	0.00	0.00	0.09	0.06	0.04	0.00	0.00	0.00	0.30	0.00	0.20

Peled	0.01	0.00	0.01	0.13	0.07	0.04	0.00	0.01	0.00	0.29	0.00	0.15
Least cisco	0.07	0.62	0.01	0.33	0.07	0.10	0.02	0.01	**2.06**	0.08	0.00	0.33
Whitefish	0.01	0.00	0.00	0.24	0.07	0.05	0.01	0.01	0.07	0.43	0.00	0.12

Table 6.

Contents of sum of eicosapentaenoic and docosahexaenoic fatty acids (EPA+DHA, mg g^{-1} of product) in cooked fish according to literature and our data. Data, obtained in this study, are given in bold.

Product	EPA+DHA	Reference
Atlantic salmon *Salmo salar* (fried)	40.1	Ansorena et al., 2010
Pacific saury *Cololabis saira* (canned)	22.7	Anishchenko et al., 2017
Atlantic salmon *Salmo salar* (fried)	19.6	Sioen et al., 2006
Pacific herring *Clupea harengus* (canned)	17.9	Gladyshev et al., 2009b
Atlantic salmon *Salmo salar* (fried)	17.0	Candella et al., 1998
Baltic sprat *Sprattus sprattus* (canned)	14.3	Gladyshev et al., 2009b
King salmon *Oncorhynchus tshawytscha* (baked)	12.4	Larsen et al. 2010
King salmon *Oncorhynchus tshawytscha* (steamed)	11.9	Larsen et al. 2010
King salmon *Oncorhynchus tshawytscha* (fried)	11.5	Larsen et al. 2010
King salmon *Oncorhynchus tshawytscha* (microwaved)	10.4	Larsen et al. 2010
King salmon *Oncorhynchus tshawytscha* (poached)	10.0	Larsen et al. 2010
Sardine *Sardine pilchardus* (fried)	8.8	Candella et al., 1998
Tugun *Coregonus tugun* (smoked)	**6.5**	**This study**
Humpback salmon *Oncorhynchus gorbuscha* (boiled)	6.0	Gladyshev et al., 2006
Brown trout *Salmo trutta* (boiled)	5.7	Gladyshev et al., 2007
Humpback salmon *Oncorhynchus gorbuscha* (stewed)	5.3	Gladyshev et al., 2006
Humpback salmon *Oncorhynchus gorbuscha* (roasted)	5.0	Gladyshev et al., 2006
Arctic cisco *Coregonus autumnalis* (smoked)	**4.3**	**This study**
Humpback salmon *Oncorhynchus gorbuscha* (fried)	4.3	Gladyshev et al., 2006
Brown trout *Salmo trutta* (fried)	4.1	Gladyshev et al., 2007
Cod *Gadus morhua* (fried)	4.1	Sioen et al., 2006
Spanish mackerel *Scomberomorus commersoni* (fried)	3.9	Candella et al., 1998
Pacific herring *Clupea harengus* (boiled)	3.9	Gladyshev et al., 2007
Pacific herring *Clupea harengus* (fried)	3.8	Gladyshev et al., 2007
Rock sole *Lepidopsetta bilineata* (boiled)	3.6	Gladyshev et al., 2007
Least cisco *Coregonus sardinella* (smoked)	**3.3**	**This study**
Rock sole *Lepidopsetta bilineata* (fried)	3.1	Gladyshev et al., 2007
Muksun *Coregonus muksun* (smoked)	**2.4**	**This study**
Cod *Gadus morhua* (boiled)	2.4	Gladyshev et al., 2007
Peled *Coregonus peled* (smoked)	**2.4**	**This study**
Cod *Gadus morhua* (fried)	2.2	Ansorena et al., 2010
Broad whitefish *Coregonus nasus* (smoked)	**2.0**	**This study**
Common whitefish *Coregonus lavaretus* (smoked)	**1.8**	**This study**
Zander *Sander lucioperca* (boiled)	1.1	Gladyshev et al., 2014
Zander *Sander lucioperca* (stewed)	1.0	Gladyshev et al., 2014
Zander *Sander lucioperca* (fried)	1.0	Gladyshev et al., 2014
Gilthead sea bream Sparus aurata (fried)	0.6	Amira et al., 2010