



## Benefit-risk ratio of food fish intake as the source of essential fatty acids vs. heavy metals: A case study of Siberian grayling from the Yenisei River

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### ABSTRACT

A formula was derived for quantification of benefit-risk ratio (hazard quotient) for the intake of a product containing essential polyunsaturated fatty acids vs. heavy metals. The quotient was used in a three year case study of the contents of essential fatty acids and heavy metals in Siberian grayling, the main food fish from the middle section of the Yenisei River. As found, in general the fish intake was potentially very beneficial for human health, except on a few occasions, when the risk outweighed the benefit because of high contents of chromium in the fish muscle tissue. The data demonstrated the necessity for regular monitoring of the hazard quotients for food fish in wild conditions, based at least on monthly sampling frequency.

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### 1. Introduction

Consumption of fish is known to provide many benefits for human health due to its high contents of essential polyunsaturated fatty acids of the  $\omega 3$  family (EFA), namely eicosapentaenoic acid (EPA, 20:5 $\omega 3$ ) and docosahexaenoic acid (DHA, 22:6 $\omega 3$ ). A regular consumption of EFA (EPA+DHA) prevents cardiovascular diseases and neural disorders (e.g. Arts, Ackman, & Holub, 2001; Garg, Wood, Singh, & Moughan, 2006; Lauritzen, Hansen, Jorgensen, & Michaelsen, 2001; Silvers & Scott, 2002). However, fish products are often contaminated by toxic organic compounds and heavy metals. Thus, recently a number of researchers have started a quantitative estimation of risks vs. benefits of fish intake for human health. For instance, Foran et al. (2005) estimated benefit-risk ratios for EFA vs. many organic contaminants and methylmercury in wild and farmed salmon. In their calculation, Foran et al. (2005) used the sum of concentrations for all of the measured contaminants, but this methodology is regarded by some authors as controversial (Hough et al., 2004).

Evidently, besides the sum data, benefit-risk ratios for EFA vs. individual organic toxic species, as well as vs. heavy metal contents in consumed fish are very desirable. Budtz-Jorgensen, Grandjean,

and Weihe (2007) developed a complicated regression model, based on dietary questionnaire responses, clinical tests and neurobehavioral tests for a separation of risks and benefits of fish intake. This very important model seems to have a further development if supplemented with data on concentrations of given beneficial and risky components, e.g. EFA vs. metals in consumed fish of different species. Different fish species from diverse locations are known to have different contents of EFA (Ahlgren, Blomqvist, Boberg, & Gustafsson, 1994; Ahlgren, Carlstein, & Gustafsson, 1999; Gladyshev, Sushchik, Gubanenko, Demirchievam, & Kalachova, 2006, 2007; Kainz, Arts, & Mazumder, 2004; Zenebe, Ahlgren, & Boberg, 1998) and contaminants, including heavy metals (e.g. Bervoets & Blust, 2003; Burger & Gochfeld, 2005; Cheung, Leung, & Wong, 2008; Gladyshev et al., 2001). Moreover, contents of EFA in the same fish species from the same location may vary significantly during a year (Ozyurt & Polat, 2006; Ozyurt, Polat, & Ozkutuk, 2005; Sushchik, Gladyshev, & Kalachova, 2007; Sushchik, Gladyshev, Kalachova, Makhutova, & Ageev, 2006), as well as, probably, metal concentrations. Data on contaminants and EFA levels in fish from particular regions could allow people to make informed decisions about which fish to eat to reduce their risk from the contaminants and increase health benefits.

Hence, the aim of our present work was to derive a simple formula for quantification of the benefit-risk ratio for the essential fatty acids vs. a given heavy metal species and to study the seasonal dynamics of this ratio for EFA and several metals on the basis of three years measurements of their contents in Siberian grayling,

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one of the main food fish of a middle section of the Yenisei River (Siberia, Russia).

## 2. Materials and methods

### 2.1. Sampling site

The Yenisei River is the eighth largest river in the world with respect to mean annual flow,  $19,800 \text{ m}^3 \text{ s}^{-1}$ , and the largest river in Russia. The main hydrochemical and ecological features of the river are given elsewhere (Telang et al., 1991; Zotina, 2008). Briefly, the main hydrochemical peculiarities of the Yenisei are a low turbidity, the saturation level of dissolved oxygen is about 100% and the content of organic carbon about  $10 \text{ mg l}^{-1}$ . The sampling site was situated in the middle section of the river, downstream of the dam of the Krasnoyarsk hydroelectric power station and upstream of Krasnoyarsk city. Detailed hydroecological description of the site is given elsewhere (Sushchik et al., 2006). Briefly, the character of the river is mountain-like, the banks are rocky and covered with taiga; current velocity is up to  $2 \text{ m s}^{-1}$  and the bottom is pebbly. Water temperature ranged within  $5\text{--}10^\circ\text{C}$  in spring–summer and  $0\text{--}5^\circ\text{C}$  in autumn–winter.

### 2.2. Fish samples

Individuals of Siberian grayling, *Thymallus arcticus* Pallas, were obtained from the catches of local anglers in the years 2005–2007. Two–four fish specimens were transported to the laboratory and sampled on the same day. Muscle tissues (fillets) below the dorsal fin were taken as the samples. Pooled samples were prepared from two–four specimens, males and females, 17–24 cm length (age 1+ – 2+). Moisture content of the tissues, 77.4%, was taken from Sushchik et al. (2007).

### 2.3. Analysis of fatty acids

Detailed description of the analysis is given elsewhere (Gladyshev et al., 2006, 2007; Makhutova, Kalachova, & Gladyshev, 2003; Sushchik et al., 2006, 2007). Briefly, lipids from samples were extracted with chloroform:methanol (2:1, v/v) three times simultaneously with mechanical homogenisation of the tissues with glass beads. Before extraction, a fixed volume of an internal standard solution (19:0) was added to the samples. Methyl esters of fatty acids (FAMES) were prepared in a mixture of methanol:sulphuric acid (50:1, v/v) at  $90^\circ\text{C}$  for 2 h. FAMES were then analysed using a GC–MS (model GCD Plus, Hewlett Packard, USA) equipped with a 30 m long  $\times$  0.32 mm internal diameter capillary column HP-FFAP. FAMES peaks were identified by their mass spectra, comparing to those in the data base (Hewlett–Packard, USA) and to those of available authentic standards (Sigma, USA). To determine double bond positions in monoenoic and polyenoic acids, GC–MS of dimethylloxazoline derivatives of FA were used.

### 2.4. Analysis of metals

The samples of muscle tissues were dried until constant weight at  $105^\circ\text{C}$ , normally within 24 h. Measurements of Na and K were made by flame photometry with FLAPHO-4 (Carl Zeiss, Jena). Ca, Mg, Fe, Mn, Zn, Cu, Ni, Pb, Co, Cr were measured by atomic absorption spectrometry using atomic absorption spectrometers AAS-1 N (Carl Zeiss) for Ca, Mg and AAS KVANT-2A (KORTEK Ltd., Russia) for Fe, Mn, Zn, Cu, Ni, Pb, Co, Cr. Instrumental detection limits for FLAPHO-4 were  $0.1 \text{ mg l}^{-1}$  ( $1 \times 10^{-5}\%$ ) for Na and for K; for AAS-1 N –  $0.1 \text{ mg l}^{-1}$  ( $1 \times 10^{-2}\%$ ) for Ca,  $0.01 \text{ mg l}^{-1}$  ( $1 \times 10^{-6}\%$ ) for Mg; for AAS KVANT-2A –  $0.01 \text{ mg l}^{-1}$  ( $1 \times 10^{-6}\%$ ) for Fe, Ni, Pb

and Co,  $0.006 \text{ mg l}^{-1}$  ( $6 \times 10^{-7}\%$ ) for Cr,  $0.003 \text{ mg l}^{-1}$  ( $3 \times 10^{-7}\%$ ) for Cu and Mn,  $0.001 \text{ mg l}^{-1}$  ( $1 \times 10^{-7}\%$ ) for Zn.

### 2.5. Calculations of hazard quotient for benefit-risk ratio

For health benefits, including preventing mental, neural and especially cardiovascular diseases, many international and national organisations (World Health Organisation, British Nutrition Foundation, The American Heart Association, etc.) have recommended daily personal consumption of about 1000 mg of EPA + DHA (will be referred to as essential fatty acids, EFA) in the human diet (e.g. Arts et al., 2001; Foran et al. 2005; Garg et al. 2006). The recommended dose of EFA for a human person is designated here as  $R_{\text{EFA}}$  ( $\text{mg day}^{-1}$ ). Thus, fish portion, FP ( $\text{g day}^{-1}$ ), which have to be consumed by a person to obtain  $R_{\text{EFA}}$  is

$$\text{FP} = \frac{R_{\text{EFA}}}{C} \quad (1)$$

where  $C$  ( $\text{mg g}^{-1}$ ) is content of EFA (EPA + DHA) in a given fish.

Consuming FP, a person will obtain a dose of a metal, DM ( $\mu\text{g day}^{-1}$ ), which can be defined as

$$\text{DM} = \text{FP} \cdot c, \quad (2)$$

where  $c$  ( $\mu\text{g g}^{-1}$ ) is content of a given metal in a given fish.

Risk of consuming a dose of metal may be characterised using a hazard quotient, HQ, (e.g. Cheung et al., 2008; Hough et al., 2004), which here is represented as

$$\text{HQ} = \frac{\text{DM}}{\text{RfD} \cdot \text{AW}}, \quad (3)$$

where RfD ( $\mu\text{g kg}^{-1} \text{ day}^{-1}$ ) is a reference dose, defined as the maximum tolerable daily intake of a specific metal that does not result in any deleterious health effects (Burger & Gochfeld, 2005; Cheung et al., 2008; Ferre-Huguet, Marti-Cid, Schuhmacher, & Domingo, 2008; Hough et al., 2004; Khan, Cao, Zheng, Huang, & Zhu, 2008; Sipter, Rozsa, Gruiz, Tatrai, & Morvai, 2008) and AW (kg) is an average adult weight. If  $\text{HQ} < 1$ , there is not an obvious risk.

Thus, substituting FP in Eq. (2) by Eq. (1) and (3) can be re-written as

$$\text{HQ}_{\text{EFA}} = \frac{R_{\text{EFA}}}{C} \cdot c \cdot \frac{1}{\text{RfD} \cdot \text{AW}} = \frac{R_{\text{EFA}} \cdot c}{C \cdot \text{RfD} \cdot \text{AW}} \quad (4)$$

Here  $\text{HQ}_{\text{EFA}}$  represents a hazard quotient for fish consumption when a human person aims to obtain from the fish the recommended dose of EFA, or, another words, risk-benefit ratio for fish consumption due to metal and EFA intake, respectively. Evidently,  $\text{HQ}_{\text{EFA}} < 1$  means the health benefit from fish consumption, and  $\text{HQ}_{\text{EFA}} > 1$  means the risk.

The Eq. (4) is partly homological to an equation of Foran et al. (2005), but Foran et al. (2005) used the sum of concentrations of all the measured contaminants, and this methodology is regarded as controversial (Hough et al., 2004). Moreover, benefit-risk ratios from Foran et al. (2005) had the dimension  $\text{g day}^{-1}$ , while  $\text{HQ}_{\text{EFA}}$  (Eq. (4)) is dimensionless.

Calculations of hazard quotients, HQ, are usually based on an average daily portion of consumed fish, determined from a statistical inquiry of population of a given territory (e.g. Cheung et al., 2008). In the suggested formula, the daily portion, FP, to be consumed by a person is determined from the physiological recommendations. Thus, the formula is general, i.e., if another dose of EFA or any other nutrient (e.g. vitamins, etc.) is recommended, the benefit-risk ratio can be easily calculated against any toxin (MeHg, PCB, etc.).

For following calculations by Eq. (4) we used  $R_{\text{EFA}} = 1000 \text{ mg day}^{-1}$ , as referred above,  $\text{AW} = 70 \text{ kg}$  (e.g. Foran et al., 2005) and median RfD values, listed in Table 1.

**Table 1**  
Values of the reference dose, RfD ( $\mu\text{g kg}^{-1} \text{day}^{-1}$ ), for some metals.

References	Mn	Zn	Cu	Cr	Ni	Pb
Burger & Gochfeld (2005)	140	300	-	-	-	-
Cheung et al. (2008)	-	15000	40	3	20	-
Ferre-Huguet et al. (2008)	24	-	40	-	20	-
Oliva, Valdes, & Mingorance (2008)	33	1000	500	-	3	-
Sipter et al. (2008)	-	300	-	-	-	35
Zukowska & Biziuk (2008)	-	-	-	-	-	3.6
Median	33	650	40	3	20	19.3

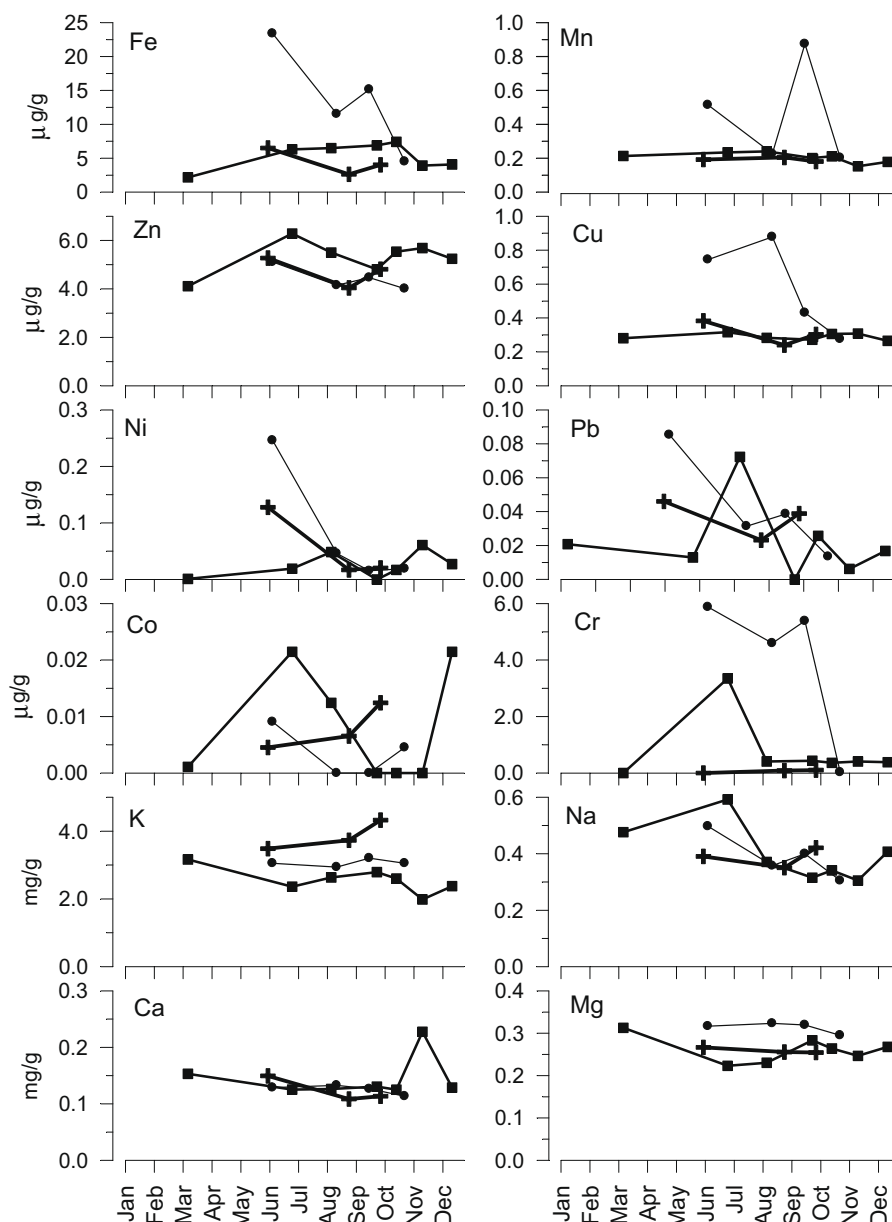
### 3. Results

Seasonal dynamics of metal concentrations in Siberian grayling are given in Fig. 1. There were no regular patterns of seasonal variations of the concentrations of any metal. However, during the study period there were significant irregular variations of the con-

centrations of Fe, Mn, Cu, Ni, Pb, Co and Cr (Fig. 1). Concentrations of the other metals, Zn, K, Na, Ca and Mg had comparatively small variations within and between years (Fig. 1).

EPA content in grayling muscle tissue peaked in September–October during all three years (Fig. 2). DHA content had no such tendency and its seasonal patterns were irregular (Fig. 2). Thus, sum content of EFA in grayling varied more than two folds within and between years, but these variations could not be strictly attributed to calendar months. On the average EPA + DHA content was  $3.0 \text{ mg g}^{-1}$  wet weight.

Values of  $HQ_{EFA}$  for all the metals, except Cr, were  $<1$  throughout all of the study period (Fig. 3). In four cases,  $HQ_{EFA}$  for Cr had values from 4.9 to 10.7, which meant deleterious health effects of intake of such fish if to obtain the recommended portion of EFA. In general, there were no regular patterns of seasonal dynamics of  $HQ_{EFA}$  values for all the metals, except Cu (Fig. 3). Values of  $HQ_{EFA}$  for Cu tended to decrease from spring to autumn (Fig. 3).



**Fig. 1.** Seasonal dynamics of concentration of metals (per wet weight) in muscle tissue of Siberian grayling from the Yenisei River upstream of Krasnoyarsk city (Siberia, Russia): circles – 2005, squares – 2006, crosses – 2007.

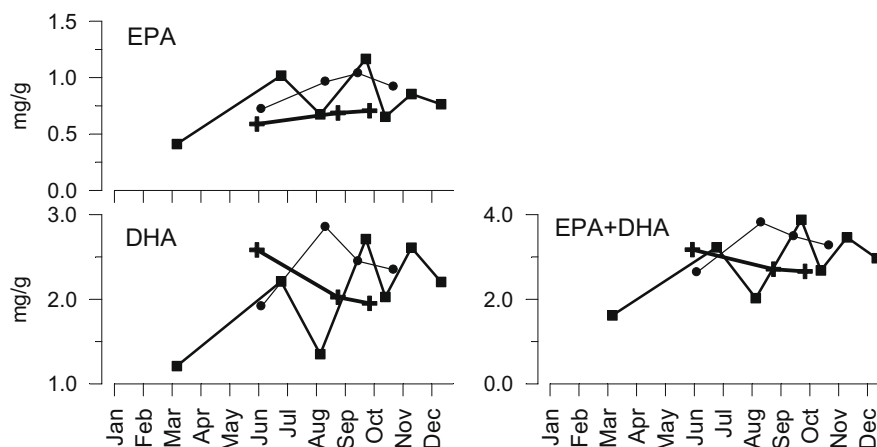


Fig. 2. Seasonal dynamics of concentration (per wet weight) of eicosapentaenoic acid, EPA, docosahexaenoic acid, DHA and their sum in muscle tissue of Siberian grayling from the Yenisei River upstream of Krasnoyarsk city (Siberia, Russia): circles – 2005, squares – 2006, crosses – 2007.

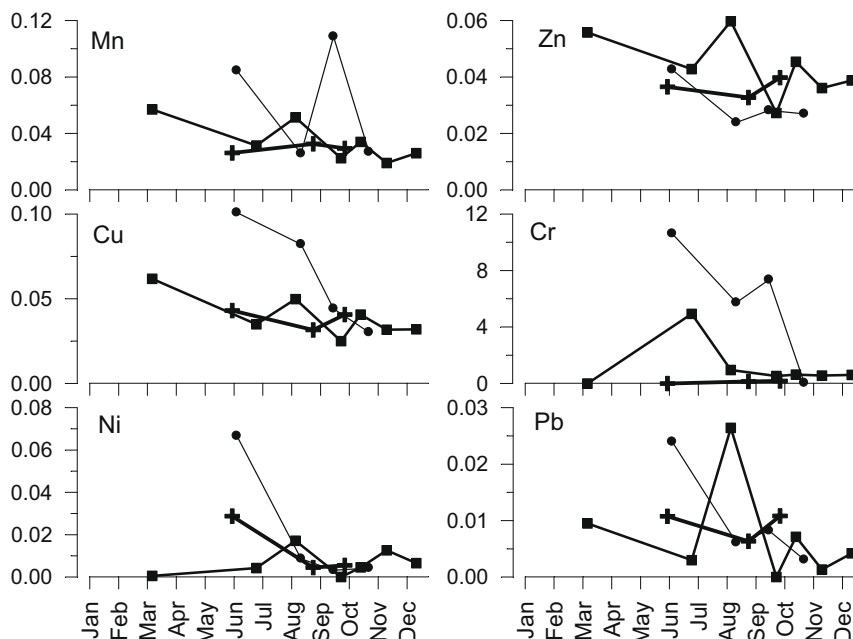


Fig. 3. Seasonal dynamics of hazard quotients,  $HQ_{EFA}$ , for benefit-risk ratio of essential fatty acids vs. heavy metals for intake of Siberian grayling from the Yenisei River upstream of Krasnoyarsk city (Siberia, Russia): circles – 2005, squares – 2006, crosses – 2007.

#### 4. Discussion

Siberian grayling had high contents of essential fatty acids, EPA and DHA (Fig. 2), which were significantly higher, than those of many common freshwater fish, such as roach, bream, carp and perch (Ahlgren et al., 1994). Compared to other Salmoniformes fish, such as European grayling (Ahlgren et al., 1994; Ahlgren et al., 1999) and rainbow trout (Kainz et al., 2004), Siberian grayling had practically the same EPA + DHA contents. Thus, Siberian grayling was found to be a valuable product for human nutrition (Sushchik et al., 2007). The contents of EFA in Siberian grayling from the studied section of the Yenisei River had some seasonal variations from  $1.62 \text{ mg g}^{-1}$  to  $3.88 \text{ mg g}^{-1}$  wet weight. As it was found earlier, spawning was the main cause of the seasonal variations of EFA contents, but the spawning cannot be attributed strictly to the calendar months since it fluctuated between years from spring to early summer and from late summer to autumn because of inter-annual variations of water discharge from the up-

stream dam of the Krasnoyarsk hydroelectric power station (Sushchik et al., 2007). Hence, a portion of the fish which should be consumed to obtain the recommended quantity of EFA also varied more than two folds. These variations evidently were of importance for calculations of the risk-benefit ratio of fish intake (Eq. (4), Fig. 3).

At present there is no uniform source of guidance or standards for most metal residues in fish tissue (Burger & Gochfeld, 2005). Nevertheless, a comparison of contents of some metals in Siberian grayling from the middle section of the Yenisei River with median international and national standards can be done. Contents of Zn in Siberian grayling, even at maximum values (Fig. 1), were about one order of magnitude lower than median international standards,  $50 \text{ } \mu\text{g g}^{-1}$  (Burger & Gochfeld, 2005), and the Russian Federal standard,  $40 \text{ } \mu\text{g g}^{-1}$  (Gladyshev et al., 2001). Cu concentrations in the grayling (Fig. 1) also were about one order lower than the Russian Federal standard,  $10 \text{ } \mu\text{g g}^{-1}$  (Gladyshev et al., 2001). Pb contents in Siberian grayling (Fig. 1) were about two orders of magnitude



lower than median international standards,  $2 \mu\text{g g}^{-1}$  (Burger & Gochfeld, 2005) and the Russian Federal standard,  $1 \mu\text{g g}^{-1}$  (Gladyshev et al., 2001). The lowest international standard for chromium is  $1.0 \mu\text{g g}^{-1}$  (Burger & Gochfeld, 2005), thus, concentrations of Cr in Siberian grayling from the Yenisei River in four cases, in June–October, 2005 and in July, 2006, were significantly higher than the standard (Fig. 1). In general, contents of many metals, Zn, Cu and Mn, in the locally important food fish, Siberian grayling, were comparable with those in many freshwater and marine fish from large world markets (Burger & Gochfeld, 2005; Cheung et al., 2008). Moreover, contents of Pb in the grayling were significantly lower, than those in commercial fish from New Jersey markets (Burger & Gochfeld, 2005) and from markets in Hong Kong (Cheung et al., 2008). Ni concentrations in the grayling were about ten times lower, than those in many freshwater and marine fish from Hong Kong markets (Cheung et al., 2008). Cr contents in many commercial fish (Burger & Gochfeld, 2005; Cheung et al., 2008) were close to those in the grayling from the Yenisei River, except the four cases in 2005 and 2006 (Fig. 1).

Hence, intake of Siberian grayling from the middle section of the Yenisei River in general appeared to be very profitable for human health due to the high EFA contents (Fig. 2) and low contents of heavy metals (Fig. 1). This statement is quantitatively supported by values of  $\text{HQ}_{\text{EFA}}$  (Fig. 3). Nevertheless, in the four cases in 2005 and 2006 the fish intake were deleterious for health because of the high contents of Cr (Fig. 3). The causes of such extremely high levels of Cr contents in muscle tissues of Siberian grayling in June–October, 2005 and in July, 2006 (Fig. 1) are not known. The studied site of the Yenisei River, located upstream of Krasnoyarsk city, is considered to be unpolluted and concentrations of all the metals, including chromium, in the water are significantly below upper acceptable limits according to Russian Federal standards, except in a few cases (O.V. Anishchenko, unpublished). At present it is not known if the occasional increases of concentrations of Cr in fish tissue are caused by anthropogenic acute events or by some geological peculiarities of watershed and natural storm events. The occasional bioconcentration or bioaccumulation of Cr in grayling muscle tissue might take place because of receiving of this metal directly from water, or through a trophic chain (Kainz, Telmer, & Mazumder, 2006). In any case, our data supported a necessity of a regular monitoring of hazard quotients and benefit-risk ratios of food fish in wild conditions, based at least on monthly sampling frequency.

A daily portion of the grayling intake to get the recommended EFA quantity,  $R_{\text{EFA}} = 1000 \text{ mg day}^{-1}$ , calculated on the basis of the data obtained in this study, was on the average about 337 g. Although this portion is significantly higher than that usually used for the risk calculations (Burger & Gochfeld, 2005; Cheung et al., 2008; Foran et al., 2005), most of the intakes during three years of study were safe ( $\text{HQ}_{\text{EFA}} < 1$ ), except the four cases for Cr (Fig. 3). Thus, consumption of Siberian grayling is profitable for humans, but levels of Cr in muscle tissue of the fish should be monitored regularly. Burger and Gochfeld (2005) pointed out that different metals resided in different fish species, and the risk information given to the public should present a complete picture to allow people choose a diversity of fish for a healthy diet. Our data on Siberian grayling support the above idea, because the level of only one metal, Cr, in the fish species occasionally provided the risk of the fish intake, while in general the intake was beneficial for health.

It is important to note, that for the above calculation of the fish intake we used EFA contents in the fresh fish. Nevertheless, as found earlier (Gladyshev et al., 2006, 2007), common ways of culinary treatment (boiling, frying, etc.) did not affect EFA content in many fish species, including those of Salmoniformes, i.e. species from the order to which grayling belongs.

Thus, using the derived hazard quotient,  $\text{HQ}_{\text{EFA}}$ , for benefit-risk ratio of essential fatty acids and heavy metals contents, it was found, that in general intake of Siberian grayling, the main food fish from middle section of the Yenisei River, potentially was very beneficial for human health, except on few occasions, when the risk overweighed the benefit because of high contents of chromium. To prevent people from deleterious effect of fish consumption and to provide healthy diets, a regular monitoring of the hazard quotients for food fish in wild conditions is very desirable.

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